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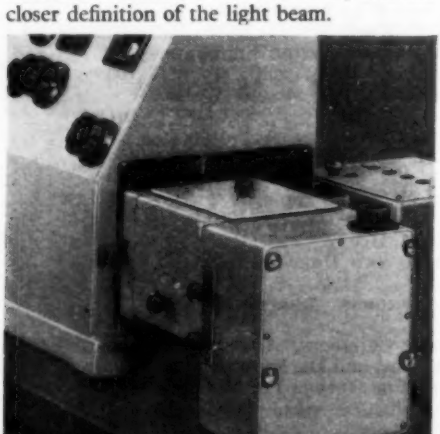

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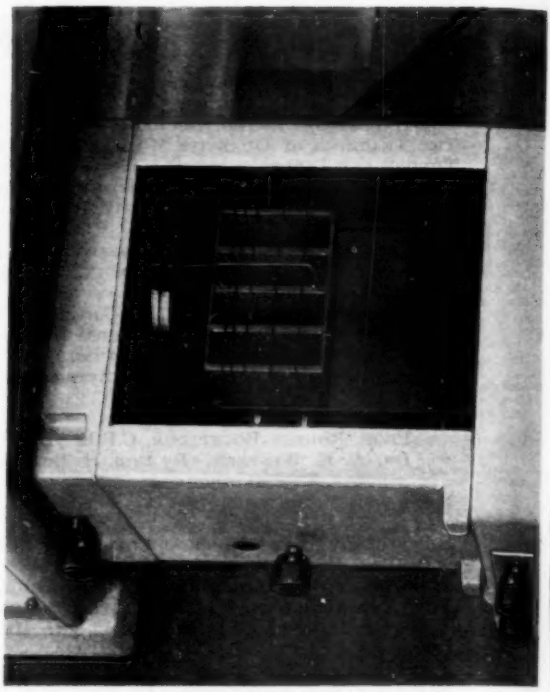

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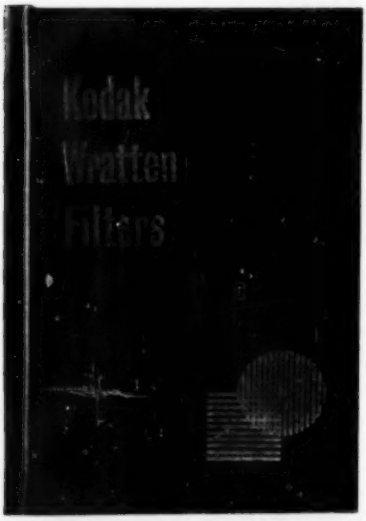

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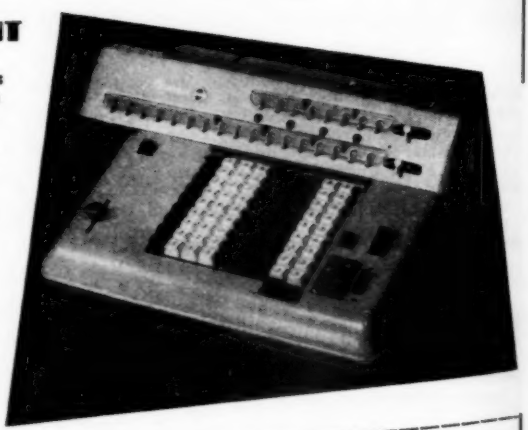

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PROFESSIONAL CIVIL SERVANTS IN BRITAIN

IN his speech at the sixteenth annual delegate Conference of the Institution of Professional Civil Servants last May, Mr. Stanley Mayne, who was then retiring from the office of general secretary, commented at some length on the position of three scientific classes in the Civil Service. He noted a feeling of frustration in the experimental officers class, and emphasized that the scientific officer class was not attracting the numbers it needed. In particular, promotion from assistant engineer to executive engineer was no longer a matter of selecting an officer best fitted for the job, but of trying to persuade someone to accept the post, because the reward for promotion was so insignificant and there was no acceptable compensation for any consequent removal and disruption of social life. Mr. Mayne also referred particularly to the position of those in high professional posts in the Service, where salaries were still disproportionate with the responsibilities and with those paid for the corresponding professional positions outside.

Mr. Mayne made it clear, in pressing these claims, particularly those of certain leading professional posts, for salaries at the same level as those of 'permanent secretaries', that the Institution of Professional Civil Servants recognized that it was not simply a matter of pay but of status also. The professional and scientific Civil Service had suffered too long because its leading members had been valued too cheaply by the Service, and because science and technology are in general valued too cheaply. With admirable restraint and a firm appeal to reason, as well as a deep sense of responsibility to the community, Mr. Mayne pressed the Institution's position, while urging that it should never forget that it was part of a larger world.

That speech alone demonstrates the existence of a very significant element of morale in the questions of salaries for professional Civil servants, and perhaps especially as regards the scientific classes. Because of that, the application of the pay-pause announced by the Chancellor of the Exchequer at the end of July has seriously strained staff relations in the Service, and Mr. Richard Nunn, who has succeeded Mr. Mayne, points out that because of the continued delay in dealing with claims submitted much earlier, professional Civil servants are beginning to believe that the Treasury is using the pay-pause not only to delay salary increases but also to stop negotiations altogether. From what he has written in recent issues of *State Service*, it appears that confidence in the staff consultation organization within the Civil Service is being seriously undermined.

Clearly questions of morale are deeply involved, and although Mr. N. Walker claims that his book, published a short time ago *, is no more than a factual study of the psychological aspects of employment as a desk worker, morale in the Civil Service can scarcely be considered in relation to one particular class of workers alone. Moreover, Mr. Walker points out at the start that his concern with morale arises from an attempt to study the efficiency of office work and to offer suggestions for improving it. This is not merely a matter of pay, of working conditions, or of improving the chances of promotion, although these can all be contributory factors, if adjustment is limited to possibilities.

Mr. Walker's book has little of direct interest to the scientist or technologist, except from the point of view of a study of human relations and of problems with which the manager of any sizable department, whether technical or not, may be confronted. He does not altogether ignore the professional classes of the Civil Service, though his questionnaire touched comparatively few of these, and only three of many professions in the Service. Among those in the small Ministry which he studied, and whose work to some extent was creative, he found a high degree of satisfaction with their work, but those in a large Ministry were much more critical of the system. Perhaps even more significant, professional workers in both Ministries showed little confidence in the administrators. Mr. Walker himself recognizes that this may be a symptom of the belief that decisions taken by laymen who are administrative colleagues or by their Ministers have too little regard to the technical advice or needs of the professional classes.

This is part of the problem of relations between the administrative and the scientific and other professional grades, and lay outside the scope of Mr. Walker's investigation. While he makes some interesting comparisons between Civil servants and the office staff of private companies to whom his questionnaire was also circulated, and discredits some common hypotheses while confirming others, it leads to little in the way of conclusions. He failed to find any reason for young male clerical officers resigning in such large numbers, or for the increase in the sick-rate with the number of workers in the office building, or to suggest any sort of training which might make office workers better at managing subordinates. On morale itself, it seems rather to raise the unanswered question: What makes work interesting?; and although the differences between the office staff in the two Ministries and in the private firms covered by Mr. Walker's investigation do not suggest that any one is preferable to the others, one important difference is again brought out which can scarcely be over-emphasized. The office worker in the Civil Service is not altogether uncritical of the Service itself and its efficiency; frequently he is critical of laxity in discipline, where

* *Morale in the Civil Service: a Study of the Desk Worker.* By Nigel Walker. Pp. viii + 302. (Edinburgh: At the University Press, 1961. Distributed by Thomas Nelson and Sons, Ltd.) 30*s.* net.

this is reflected in inefficiency. The Service, however, is highly sensitive to criticism from outside, especially ill-founded or ill-informed criticism, and the bearing of this on morale and efficiency in the Service generally is possibly closer than Mr. Walker himself indicates. It could well encourage the group loyalty or *esprit de corps*, often so important in efficiency, to lead to a blindness to faults which undermines efficiency in other ways.

Apart from the comments on the need for restraint and fairmindedness in public criticism, the two most important points made in Mr. Walker's book are the reminders that we need to know much more about the causes which lead to satisfaction in work and also about the relations between the professional and administrative officers. There is still the feeling that the work of the former is neither properly valued nor clearly understood, and that Mr. Walker directs even passing attention to this serious problem is sufficient reason for the scientist and technologist to take note of his book.

While those professional Civil servants who occupy very senior positions are given both status and remuneration more in accordance with their responsibilities, as is shown by the annual lists of special promotions, there must be many others whose conditions of enployment could be much improved. Perhaps Mr. Walker's book might be put in the hands of the administrative classes of the Civil Service, where its readers would learn something of the outlook of their opposite numbers on the scientific side, to the ultimate benefit of the whole of the Service.

THE HALLEY BAY EXPEDITION OF THE ROYAL SOCIETY

The Royal Society International Geophysical Year Antarctic Expedition

Halley Bay, Coats Land, Falkland Islands Dependencies, 1955–1959. General Editor: Sir David Brunt. Vol. 1: Introductions, Aurora and Airglow, Geomagnetism. Pp. xv + 405 + 12 plates. 140*s*.; 23 dollars.

THE origin, organization and execution of the project known as the International Geophysical Year, and in particular the participation of the United Kingdom, are well known[1]. Full details concerning the Royal Society's International Geophysical Year Expedition to Halley Bay, Antarctica, during 1955–59, are given in the introduction by Sir David Brunt, former Physical Secretary of the Royal Society, to the volume under review. The British National Committee for the International Geophysical Year was set up by the Council of the Royal Society on December 11, 1952, and formal approval was given by this Committee on March 1, 1955, to the general proposals for setting up a station in the Antarctic. The necessary support from H.M. Treasury and the Colonial Office was forthcoming, and the advance party, with Lieutenant-Commander D. G. Dalgleish as leader, left Southampton on November 22, 1955; a landing was made on January 11, 1956, at a suitable point *c.* 75° 31′ S., 26° 37′ W., which was later named Halley Bay.

The main party left England during November 1956 under the leadership of Colonel R. A. Smart, in the M.S. *Magga Dan*, along with the main party of the Commonwealth Trans-Antarctic Expedition. The Antarctic Sub-committee set up by the British National Committee had planned in detail a programme of observations on meteorology, geomagnetism, aurora and airglow, ionospheric physics, glaciology and seismology, including an extensive programme of radio-astronomical observations. A further visit was made to the base during the Antarctic summer 1957–58, when Colonel Smart, following his wish expressed at the time of his appointment in 1956, returned home and was succeeded as leader by J. MacDowall.

An outstanding criticism of the International Polar Year expeditions of 1882–83 and 1932–33 had been the long delay in publishing some of the observations. This consideration was doubtless in the minds of the Antarctic Analysis Subcommittee set up to supervise the publication of the scientific results of the present expedition, and it is commendable that Volume 1, dealing with aurora and airglow, and geomagnetism, should have appeared so soon. The observations themselves are preceded by a vivid narrative account of the establishment and maintenance of the base; this account is illustrated by a series of excellent photographs, which in particular show the measures taken to combat the accumulation of snow, amounting even on a flat drift-free surface to 3 ft./year.

The visual and photographic observations of the aurora are discussed by S. Evans and G. M. Thomas; an all-sky camera was used whenever conditions were favourable. Some very beautiful photographs, together with a table of movements of auroral features, are given in this volume. An airglow photometer, sensitive to a band of infra-red wave-lengths of 7,400–11,300 Å., was operated as conditions permitted; the report by G. M. Thomas, who was observer of auroral and airglow phenomena to the main party, lists the mean nightly airglow intensities, which constitute a valuable record inasmuch as it was possible in general to recognize those occasions on which the measured zenithal intensity was enhanced by light from auroræ.

The geomagnetic observations occupy by far the greater part of the volume. The laboratory was a non-magnetic hut with its centre line along the observed magnetic meridian; it was at least 110 m. from any large ferrous objects, and tractors were not allowed within 100 m. of the hut. The observatory was built on a floating ice-shelf which moved slowly and steadily seaward. The foundations for the recording and absolute instruments were wooden piles driven 6 m. into the snow. Continuous registration of the horizontal force (H), the declination (D) and the vertical force (Z) were made by two independent recorders of the La Cour type. The absolute instruments were three quartz-fibre horizontal-force magnetometers, two balance magnetometers for vertical force, and a portable unifilar magnetometer-inductor. The discussion by J. MacDowall and A. Blackie of the geomagnetic work is too detailed to summarize here. It contains the diurnal variation (including a harmonic analysis of H, D and Z), a detailed examination of K-indices, Q-indices and the absolute daily range. Diagrams are given of the vector field changes, and tables are included listing (*inter alia*) the hourly mean values of the three components of geomagnetic force for every day, the 3-hourly K-indices, and the Q-indices for consecutive

intervals of 15 min. The volume concludes with reproductions of the complete series of magnetograms from April 30, 1957, to December 31, 1958.

In all, this work presents the results of a prodigious amount of labour. It is well set-out, and the typography is excellent. The Royal Society and the authors are to be congratulated on their fine achievement.

R. STONELEY

[1] *Nature*, **188**, 529 (1960).

THE TEACHING OF QUANTUM MECHANICS

Introduction to Quantum Mechanics

By Prof. Robert H. Dicke and Dr. James P. Wittke. Pp. xi+369. (Reading, Mass., and London: Addison-Wesley Publishing Company, Inc., 1960. 66*s*.)

Problems in Quantum Mechanics

By I. I. Gol'dman, V. D. Krivchenkov, V. I. Kogan and V. M. Galitskii. Pp. iii+394. (London: Infosearch Limited, 1960. Distributed by Cleaver-Hume Press, Ltd.) 42*s*.

Problems in Quantum Mechanics

By I. I. Gol'dman and V. D. Krivchenkov Edited by Prof. B. T. Geilikman. Translated from the Russian by E. Marquit and E. Lepa. Authorized revised edition. Pp. viii+275. (London and New York: Pergamon Press, 1961.) 50*s*.

AFTER many years of famine in the supply of quantum mechanics text-books, there is now an abundance and more titles keep appearing. It is possible therefore to be fairly critical in deciding which text-books to recommend to students. Quantum theory is gradually taking a larger part in undergraduate training in general and theoretical physics, and the typo of book required at the undergraduate level is not, for example, Dirac's, which is too difficult, or that of Landau and Lifshitz which is too big and thorough.

Prof. Dicke and Dr. Wittke have written their text for the first postgraduate physics year at Princeton, and it covers much the same ground as that at many British universities (it should be all), in the third year of honours physics. All the essentials of quantum theory are covered, and the late chapters of the book cover the effects of strong fields on atoms, scattering theory, the effect of statistics on the two and many-body systems, and on quantum statistical mechanics. There is a surprising and serious omission from the contents since the uncertainty relation between energy and time is never mentioned. On the whole it is well written, with the difficulties that a beginner experiences well aired. Useful sets of examples are appended to each chapter. But it can be criticized in that several mathematical statements are not precise, and the same lack of precision shows up in several of the discussions of points of principle. For example: Why should Hermites polynomials have to terminate? Why should they be polynomials at all? Why should the reader accept, without proof, that $(\exp ikr)/r$ is the Green function for scattering and not $(\exp -ikr)/r$? The discussion of orbital and spin angular momentum make these appear only trivially different, and in discussion of what Dirac calls the Schrödinger and Heisenberg 'pictures', the word representation is confusingly used to mean picture as well as its mathematical meaning with respect to a basis. There are several other minor inaccuracies and obscurities, and a general tautening up of statements could be made throughout the book without making it longer or more difficult. In spite of these drawbacks it is an attractive book and beautifully produced.

The two collections of examples present a curious situation for they are effectively the same book, being translations of a set of problems with full solutions, used by Drs. Gol'dman and Krivchenkov in teaching a course of quantum mechanics based on Landau and Lifshitz's text. The volume from Infosearch has added further problems from a comparable set by Kogan and Galitskii, while that from Pergamon is based solely on Gol'dman and Krivchenkov, though from an extended and revised version. The latter states it is the 'authorized version', which may account for the price difference. As it stands the Infosearch edition is considerably larger and clearly the 'best buy'. These problems cover much more ground than say Dicke and Wittke, and are suitable for theoretical specialists. With the book of Landau and Lifshitz they throw up a powerful challenge. This is how quantum mechanics is taught to theorists in Moscow. Are British theorists taught as thoroughly? Moreover, in those universities where they are, do the students also reach the same standard in hydrodynamics, physical kinetics, elasticity, etc., as the introductions to the Landau and Lifshitz texts assure us the Russian theorists do. Regretfully one must give the answer that scarcely anywhere in Britain is this standard aimed at, let alone achieved.

S. F. EDWARDS

THEORY AND TABLES OF THE BLUE SKY

Tables related to Radiation emerging from a Planetary Atmosphere with Rayleigh Scattering

By Kinsell L. Coulson, Jitendra V. Dave and Zdeněk Sekera. Pp. x+548. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1960.) 116*s*. net.

ONE of the best-known of the many fundamental contributions to science made by Lord Rayleigh during his long career was his discovery of the law of molecular scattering (named after him) and its application to the explanation of the colour and polarization of the sunlit sky. The problem of the diffuse reflexion and transmission of sunlight in an atmosphere scattering it in accordance with Rayleigh's law has since become one of the classical problems of mathematical physics, of particular interest to meteorology. Many efforts were made toward its solution—culminating in the well-known work by Chandrasekhar, who succeeded in constructing its exact solution in terms of the so-called X- and Y-functions satisfying four pairs of simultaneous integral equations—but, alas, these equations turned out to be non-linear and of the type which resolutely defies any possibility of an analytical solution.

Yet, in order to learn what these functions are capable of revealing, it is necessary to evaluate them numerically and insert in various relevant expressions. The construction (by successive approximations) of numerical solutions of the respective equations has indeed been undertaken recently by the authors of

the volume under review, on an impressive scale, using the facilities of the Watson Scientific Computing Laboratory in New York and of the Institute for Numerical Analysis of the U.S. National Bureau of Standards at Los Angeles. From the functions X and Y so obtained, the Stokes parameters of the radiation emerging in different directions at the top and at the bottom of the atmosphere have been computed for different elevations of the Sun and different reflectivities of the ground. The results of these computations, performed by the authors at the Data Processing Centre of the University of California at Los Angeles, are then presented in this book.

The volume itself consists largely of an impressive set of tables, containing accurate data which should meet any conceivable problem encountered in the field of atmospheric optics for a long time to come, and represents a permanent contribution to the literature on physical meteorology. We can indeed agree with Chandrasekhar's words in the foreword to this book that ". . . with the publication of this volume, comprising the work of Dr. Sekera and his collaborators, the problem that was formulated by Rayleigh in 1871 has now at last found its complete solution".

ZDENĚK KOPAL

STRUCTURE, LIFE-HISTORY AND DISTRIBUTION OF *GNETUM*

Gnetum

By P. Maheshwari and Vimla Vasil. (Botanical Monograph No. 1.) Pp. xii+142. (New Delhi: Council of Scientific and Industrial Research, 1961.) Rs. 20.00; 40*s*.

GNETUM is a genus consisting of rather more than thirty species of which most are woody climbers, but some are shrubs and small trees. It is confined to certain humid regions in South-east Asia, West Africa and the north-eastern parts of South America. It was at one time classified with *Ephedra* and *Welwitschia* in the order Gnetales; but it is now generally accepted that these last two genera are not very closely related to *Gnetum*, so that the order Gnetales is now thought of as consisting of this one genus. The chief botanical interest of the genus is that all its members exhibit, in their floral morphology, embryology and in the structure of their vegetative organs, a mixture of angiosperm and gymnosperm characters. In consequence the genus has been much discussed whenever the origin of the flowering plants is under consideration. To those botanists who are familiar with *Gnetum* mainly on account of its phylogenetic interest it may come as a surprise to learn that *Gnetum gnemon* is cultivated for the sake of its edible seeds, that fibre from the bark is used to make fishing nets and ropes, and the wood of old trees is used in cooperage.

The book under review is the first of a series of botanical monographs that is to be produced by the Indian Council of Scientific and Industrial Research. The senior author, who is professor of botany at Delhi, is already well known for the extensive programme of research carried on in collaboration with his pupils and associates, on the morphological aspects of the embryology of the flowering plants. His *Embryology of Angiosperms* is also much used as a work of reference and by students. In the monograph now under review the authors integrate and summarize all that has been done on the geographical distribution, morphology, anatomy and embryology of *Gnetum*. The relationships of the genus to the angiosperms and gymnosperms and also with *Ephedra* and *Welwitschia* are also fully discussed. The book is well illustrated by distribution maps for the species, as well as by line drawings and photographs, all of which are of excellent quality and well reproduced, many of these illustrations being original. There are a comprehensive bibliography and a single index to authors, plant names and subjects.

The book, which is well printed and reasonably well bound, can be recommended to research workers, teachers and students who will no doubt welcome this modern treatment of a topic that has been of interest for many years. Readers will find, however, that the authors are cautious in drawing phylogenetic conclusions, for the text ends with these words: "Thus *Gnetum* remains largely a phylogenetic puzzle. It is gymnospermous, but possesses some strong angiospermic features".

C. R. METCALFE

WORLD FOOD PROBLEMS

World Review of Nutrition and Dietetics

Vol. 2. Edited by Geoffrey H. Bourne, assisted by Eileen M. H. Wilson. Pp. viii+245+14 plates. (London: Pitman Medical Publishing Co., Ltd., 1960.) 60*s*. net.

THE second volume in this series is slightly shorter than the first, but, as the editor remarks in the preface, it "spreads its international net even wider". There are two contributions from the United States and one each from France, Sweden, South Africa, New Zealand, Switzerland and the United Kingdom. The subject-matter again covers a wide range, as the titles of the following four contributions out of a total of nine indicate: "Proteins and Hæmatopoiesis"; "The Effect of Malnutrition on the Eye, with Special Reference to Work with Experimental Animals"; "The Microbiology of Digestion"; "The Role of Carotene and Vitamin A in Animal Feeding". The reviews are written by well-informed and experienced workers, who between them list some 2,000 references.

In the preface to Volume 1 the editor expressed the hope that each review would "provide a critical evaluation of the field" and added that "the author's interpretation, his views and his theorising will be welcomed as an integral part of his article". These aims have not been fully achieved. Some of the authors draw some conclusions from their extensive reviews; other confine themselves to a survey of the literature which ends abruptly without summary or comment. It would surely be useful if authors of multi-reference scientific reviews would attempt more often to summarize the salient points and briefly discuss the key question: Where do we go from here? This might help towards discarding literature which has contributed at some stage to the advance of knowledge, but no longer needs to be consulted.

With this second volume, the *World Review of Nutrition and Dietetics* is fully launched and on the way to establishing itself as a publication of importance to research workers in nutrition and associated fields throughout the world. It is well printed and produced. The editor is to be congratulated on the high standard attained.

W. R. AYKROYD

Biological Education in American Secondary Schools, 1890–1960

By Paul De Hart Hurd. (Biological Sciences Curriculum Study Bulletin, No. 1.) Pp. ix+263. (Washington, D.C. : American Institute of Biological Sciences, 1961.) 4.75 dollars.

TO help in the preparation of new material for American schools, Dr. Paul De Hart Hurd of Stanford University was invited to make a historical critique of the development of secondary school biological education in the United States. His report describes the investigations that he has made of the teaching of biology in the American high school covering the period 1890–1961, and is limited to consideration of curriculum development and investigations of classroom and laboratory learning. Training of teachers, professional training of biologists and special programmes for high-school students are not considered. The work would be of interest to students of the history of biological education. Teachers of biology in Britain could find many parallels to the questions which Dr. Hart Hurd raises about contemporary teaching in the United States ; they would also wish that some of the answers had been forthcoming.

Histologie und Mikroskopische Anatomie des Menschen

Von Prof. W. Bargmann. Dritte, verbesserte Auflage. Pp. xvi+820. (Stuttgart: Georg Thieme Verlag, 1959.) 69.60 D.M.

THIS is the third edition of Prof. Bargmann's monumental work, which originally appeared in two volumes. In keeping with—as the author says—"the renaissance in structural biological research" which we witness at the present time, the original text has been revised in many respects. This is particularly so in the chapters dealing with cells and tissues generally. Accounts of ultra-structure as revealed by electron microscopy have been added *per se* or skilfully woven into the text. The neurone theory has been restored to its appropriate place, full descriptions are given of the fine structure of synapses, sensory epithelia, Ranvier's nodes, motor end-plates and of the myelin sheath. The reality of bile capillaries and Disse's space is attested and the 'sex chromatin' fully discussed, to mention only a few items; but 'amitosis' as a mode of cell proliferation still looms unduly large. With the fast advance being made in the field of electron microscopy, keeping up to date is almost impossible, but it is regrettable that some of the exciting and illuminating pictures which have been obtained with the electron microscope of skeletal muscle are not included.

Where the book scores over the many others available in the field of histology is in the treatment of the microscopic anatomy of the connective tissue apparatus, its intimate architecture and the relationship of particular designs to the function of individual organs. First-class descriptions are given of complex mechanical, elasto-muscular arrangements and, in some instances, also of intricate vascular patterns.

The compass of the book being exceptionally wide, it includes many embryological data. The value of these could be enhanced by some more illustrations of what are, after all, very complex situations and events, and, sometimes, a single picture would aid and clarify a very condensed description. Similarly, the chapter on the central nervous system would gain by additional illustrations. Valuable information is given on post-natal developmental processes and on senile changes.

The masterly style bears the stamp of the dynamic personality of the author, makes fascinating reading and brings microscopic anatomy very much to life. There can be no doubt that the interest of the student must be aroused and highly stimulated by such a vivid account; at the same time, the student would do well to keep an open mind towards many problematic issues which have remained somewhat hidden in this presentation. Key references to the literature are given at frequent intervals; and photographs of famous microscopists and brief footnotes here and there direct attention to historical aspects of the subject.

F. JACOBY

Coir

Its Extraction, Properties and Uses. Pp. v+54. (New Delhi: Council of Scientific and Industrial Research, 1960.) Rs. 6.00; 10*s*.

THIS small book of some fifty pages gives a good account of the Indian coir or coconut fibre industry, India being one of the main producers of this important commercial fibre. The book is the result of a decision made by the All India Board of Technical Studies in Textile Technology in 1950 to collect and collate available information on the coir industry. This has now been presented in condensed form under such headings as: extraction of coir, structure and properties of the fibre, spinning, weaving, dyeing and printing, ropes and cordage, other uses, marketing and trade.

The value of coir as a fibre lies in its resilience or elasticity and its durability and damp resistance, properties which render it so well suited for matting or floor-covering and certain types of cordage. One of the features of its production is the traditional retting process with the drawbacks (evil smell) that go with it. It also involves a considerable loss of potential plant food (potassium and phosphorus). Reference is made to various alternative methods of extracting the fibre from the husk, mechanical and chemical, that have been put forward from time to time. For various reasons, however, the old method prevails in what is still essentially a cottage industry in India. A number of interesting photographs and a short bibliography are included.

Indian Tobacco

A Monograph. Pp. xxii+413+154 photographs. (Madras: Indian Central Tobacco Committee, Ministry of Food and Agriculture, Government of India, 1960.) Rs. 36.00; 72*s*.; 10 dollars.

THIS book, which contains a great deal of useful information on the cultivation, production and use of tobacco in India, owes its origin to the Indian Central Tobacco Committee and is the work of several authors, including past and present directors of tobacco research. As the Indian Minister for Agriculture points out in his foreword, India is faced with a land shortage or crisis, with simultaneous pressure from food and non-food crops. Tobacco falls into the latter category and cannot claim priority. Steps to increase yield from the existing acreage are therefore all the more imperative. This comprehensive work should play a useful part in this connexion.

Tobacco cultivation in India differs from that in most other important tobacco growing countries because in India the crop has to be grown under monsoon conditions. The book may be of special

value to those concerned with tobacco cultivation in other parts of the world where monsoon or similar climatic conditions prevail. It is divided into three parts. In the first part soils, climate, botany, cultivation, curing, pests and diseases, etc., are dealt with. Part 2 deals in detail with the five basic types of tobacco produced in India, namely, flue-cured tobacco, bidi tobacco, cigar and cheroot tobacco, hookah tobacco, chewing and snuff tobacco. The third part is concerned with marketing.

The book is not without certain errors, one of the most lamentable being the statement in the second paragraph of the introduction that Christopher Columbus discovered tobacco in his voyage from Persia to Spain on the island of Tobags!

Transactions of the Society of Rheology

Vol. 5, 1961. Edited by E. H. Lee. Pp. ii+382. (New York: Interscience Publishers Inc.; London: Interscience Publishers, Ltd., 1961.) 10.50 dollars.

THE 1961 volume comprises the majority of the papers presented at the thirty-first annual meeting of the (American) Society of Rheology in Pittsburgh (Pa.) and includes symposia on the mechanics of continua and on the rheology of suspensions as well as nearly twenty papers concerned with other topics. I remember criticisms at the first meeting (1929) that rheology was too mathematical for the practical man; but, more than thirty years later, it seems that workers in many industries are quite able to cope with triple integrals and tensor notation.

Like all sciences, rheology has its fashions. At the moment, normal stresses ('Weissenberg effects') and dynamic mechanical properties lead in the theoretical field; and die entry and extrusion problems on the practical side. High polymers are still in the lead among the materials of interest, but a paper on the rheology of blood reminds us of increasing attention to biological applications.

The Bingham Medal for 1960 has been awarded to Dr. B. H. Zimm for outstanding work linking optical with rheological methods in the study of macromolecules and the determination of their molecular weights.

Many of the papers are very short, but the authors' addresses are given and readers will doubtless write for reprints of longer articles.

The *Transactions* are, as always, well printed and produced but the price for a volume of this size is rather high. G. W. Scott Blair

Technical Data on Fuel

Edited by H. M. Spiers. Sixth Edition, completely revised. Pp. xi+360. (London: British National Committee, World Power Conference, 1961.) 60s. net.

THIS sixth edition of a well-known and much-used reference book constitutes a marked advance on its predecessors, for it contains more information in the form of both text and tables and the size of the page has been increased. This may be a disadvantage to the worker who wishes to have a readily portable book of useful handiness, but the greater wealth of information compensates amply for the change in character. The tables and diagrams are brought into better relation with the text and most of the tables are placed on single pages. In general it will be agreed that the changes will be approved by fuel technologists. The contents of the book have been extensively revised, tables have been recalculated where necessary, or replaced on the basis of more recent or more trustworthy data, and the conversion factors and the symbols for units have been brought into conformity with British Standard specifications. These features that have been confirmed in use constitute a worthy improvement. The sections that have been extensively revised or completely re-written include many subjects in which technical advances are bound to move with the times, and the new information is therefore all the more welcome.

Of the entirely new topics included in this new edition mention should be made of information relating to atomic and nuclear fuels, the viscosity blending of oils and flow in fluidized systems. A table of additive viscosity functions derived from the usual type of viscosity blending chart enables the viscosity of a blend of several fuel oil components to be estimated with considerable accuracy. Since the use of fluidized beds is coming more into use in fuel technology the information provided is typical of the general character of the advancement shown in this edition. The general legibility of the text, charts and tables is a noteworthy feature of the book.

R. J. Sarjant

Symposium on Pilot Plants in Metallurgical Research and Development

Held at the National Metallurgical Laboratory, Jamshedpur, India, February 15th to 18th, 1960. Edited by R. M. Krishnan. (Organized by the National Metallurgical Laboratory, Jamshedpur.) Pp. 304. (Calcutta: The Technical Journals of India Private, Ltd., 1960.) n.p.

THIS symposium drew a large gathering of scientists and metallurgists from ten overseas countries as well as equally important representatives from metallurgical science and industry from India. Formal papers presented totalled 29, of which 16 were by foreigners and 13 by Indians. There were also three lectures by visitors. All papers and lectures are reprinted in full together with the discussions.

The National Metallurgical Laboratory at Jamshedpur is concerned with ferrous and non-ferrous problems, and while part of its activity is devoted to laboratory research in order to exploit to the full the mineral resources of India, a major emphasis has been placed on the design, erection and operation of pilot plants. The scope of these pilot plants ranges from the basic chemistry of metals to financial evaluations of ambitious metallurgical processes. Many of these experiences were reported.

Some of the papers directed attention to the uses and limitations of pilot plant studies and others described in detail many of the successful pilot plants operating in the world. These included coking studies, cupola and blast-furnace operation, ore sintering, oxygen steel-making, refractories, continuous casting, mineral dressing, etc.

It is clear from the papers and discussion that most people present fully appreciated the gulf which exists between initial laboratory findings and economically attractive commercial operations. They also realized the vital part that well-planned pilot plant investigations can play in bridging this gap. This book forms a valuable collection of experiences on this topic and covers a wide range of industries.

E. W. Voice

ANALYSIS OF THE RELATIONSHIP BETWEEN DEPOSITION OF LONG-LIVED FISSION PRODUCTS AND LEVEL OF ACTIVITY IN MILK

By Dr. W. ANDERSON, L. K. BURTON and J. O. CROOKALL

Physics Department, Institute of Cancer Research: Royal Cancer Hospital, London, S.W.3.

IN recent years attention has been focused on the mechanisms of transit of fall-out radionuclides in food chains, particularly the steps leading to the contamination of milk. One of the main issues to be resolved is the extent to which the levels of activity in foods are determined, on one hand, by the deposit of activity accumulated over the years in the top of the soil, and, on the other hand, by the proximate deposition. The evidence available on strontium-90 and cæsium-137 concentrations in milk produced in Great Britain indicates that, up to the present time, the activity-levels have been determined largely by the rate of fall-out at or near the time of production of milk[1–4]. However, the relative importance of the contributions of activity from the cumulative and current deposits may be expected to change with the changing fall-out conditions, and it would therefore be useful to obtain a more quantitative expression of these relationships than is available at present. Since marked changes in the rate of fall-out occurred during the period 1958–60, it is now possible to tackle this problem by correlating the levels of activity for milk, for various open-pasture feeding months, with the corresponding cumulative and current amounts of deposition.

In the present article we report the levels of strontium-90 and cæsium-137 occurring in milk produced in west Cardiganshire, Wales (Felinfach creamery) throughout 1960, and examine these results in relation to the situation for the same series of samples in 1958–59. It should be noted that the activity values reported here refer to levels in milk produced in a single area and they should not be taken to represent the average levels for Britain as a whole. On the other hand, except in the case of special areas where the rate of growth of herbage is abnormally low[4], the variation in levels of activity for milk between different regions of the country is largely attributable to difference in rainfall[1–3,5]. This indicates that the relationships between levels of activity for milk and deposition-levels in the different regions are broadly similar in nature to those discussed below for the Felinfach region.

The milk sampling and measuring techniques used in this work have already been described[3,6,7]. Samples of dried skimmed milk, each representative of a total day's production, were obtained at weekly intervals, and measurements were made on bulked samples, compounded from four consecutive weekly samples. Thus, each measured value represents approximately the mean level of activity in the milk drawn from the Felinfach area, averaged over a 4-weekly period. It may be noted that the full open-pasture feeding period in the area extends from May to October

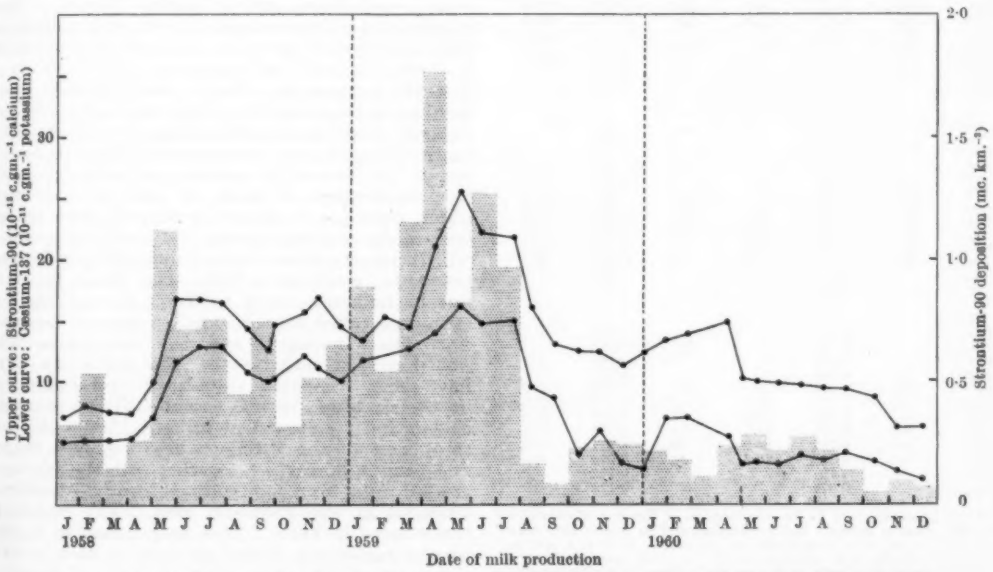
Upper curve: Strontium-90 (10^{-12} c.gm.$^{-1}$ calcium)
Lower curve: Cæsium-137 (10^{-11} c.gm.$^{-1}$ potassium)
Strontium-90 deposition (mc. km.$^{-2}$)
30
20
10
2·0
1·5
1·0
0·5
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1958
1959
1960
Date of milk production

Fig. 1. Levels of strontium-90 (upper curve) and cæsium-137 (lower curve) in milk from Felinfach, west Cardiganshire, Wales (1958–60). The histogram shows the monthly deposition of strontium-90 in the Felinfach area (ordinate on right)

Table 1. Mean Levels of Strontium-90 and Cæsium-137 in Felinfach Milk during Successive May–October Periods (1958–60)

Year	Strontium-90 (μμc./gm. calcium)	Cæsium-137 (μμc./gm. potassium)	Cæsium-137/strontium-90 (μμc./l./μμc./l.)
1958	15·2	114	10·0
1959	18·5	113	8·1
1960	9·5	36	5·0

inclusively. The results for strontium-90 and cæsium-137 levels in the milk during 1958–60 are presented in Fig. 1, together with a histogram showing the monthly strontium-90 deposition in the Felinfach area. The trend of the monthly cæsium-137 deposition was broadly similar to that shown for strontium-90, with an average cæsium-137/strontium-90 ratio of 1·7.

Looking at the 1960 results (Fig. 1) it will be seen that the levels of both strontium-90 and cæsium-137 in the milk rose during the early months of that year. This reflects the change-over to winter feeding conditions which involved the use of feeding stuffs harvested during a period of relatively high fall-out rate in 1959. When we come to the 1960 open-pasture feeding period (May–October) there is a sharp change to lower steady values. In Table 1 the average level of activity in the milk during the May–October period of 1960 is compared with those for the corresponding periods of 1958 and 1959. It will be seen from Table 1 that the level during the 1960 open-pasture period was markedly lower than those for the two preceding years. A glance at Fig. 1 shows that the trend of the levels of activity for milk was broadly similar in direction to that of the rate of deposition. In contrast, the cumulative total of deposition rose by a factor of 1·75 between mid-1958 and mid-1960 (Table 2). Thus, the view[3] that the sharp increases of activity in the milk during 1958 and 1959 were due chiefly to corresponding rises in fall-out rate, is strongly confirmed.

Table 2. Data on Strontium-90 and Cæsium-137 Levels in Milk Samples and on Rate of Deposition and Cumulative Deposition in the Felinfach Area during 1958–60

Month*	Strontium-90: Activity in milk (10^{-12} c.gm.$^{-1}$ calcium)	Strontium-90: Rate of deposition (mc. km.$^{-2}$ mth.$^{-1}$)	Strontium-90: Cumulative deposition (mc. km.$^{-2}$)	Cæsium-137: Activity in milk (10^{-12} c.gm.$^{-1}$ potassium)	Cæsium-137: Rate of deposition (mc. km.$^{-2}$ mth.$^{-1}$)	Cæsium-137: Cumulative deposition (mc. km.$^{-2}$)
1958						
Jan.		0·328			0·623	
Feb.		0·506			0·961	
Mar.		0·148			0·251	
April		0·212			0·360	
May	13·5	1·070	13·9	95	1·819	25·8
June	17·2	0·707	14·6	123	1·273	27·0
July	16·3	0·727	15·3	135	1·236	28·2
Aug.	15·1	0·443	15·7	113	0·797	28·9
Sept. +	13·8	0·719	16·4	103	1·079	29·9
Oct. +	15·2	0·294	16·6	112	0·529	30·4
Nov.		0·492			0·738	
Dec.		0·663			0·995	
1959						
Jan.		0·900			1·620	
Feb.		0·451			0·586	
Mar.		1·153			1·960	
April		1·690			2·873	
May	25·5	0·834	22·5	161	1·168	39·9
June	22·2	1·190	23·7	147	1·547	41·3
July	21·8	0·852	24·5	149	1·363	42·6
Aug.	16·0	0·156	24·6	96	0·312	42·8
Sept.	13·0	0·073	24·6	87	0·073	42·8
Oct.	12·5	0·227	24·8	40	0·431	43·2
Nov.		0·257			0·488	
Dec.		0·246			0·517	
1960						
Jan.		0·219			0·372	
Feb.		0·198			0·376	
Mar.		0·105			0·147	
April		0·243			0·413	
May	10·0	0·284	26·0	35	0·483	45·3
June	9·8	0·211	26·1	32	0·317	45·6
July	9·7	0·256	26·4	39	0·384	45·9
Aug.	9·4	0·211	26·5	35	0·338	46·1
Sept.	9·2	0·132	26·6	40	0·211	46·2
Oct.	8·7	0·059	26·6	35	0·094	46·2
Nov.		0·101			0·162	
Dec.		0·071			0·114	

* The time unit used in the computation of the rate of deposition was the calendar month. The periods covered by five of the bulked milk samples in their original grouping (refs. 2, 3) (Fig. 1) did not correspond closely to a calendar month. In these cases we have regrouped the single samples and have made fresh determinations. In two cases, indicated by +, the bulked samples each comprised only two single samples.

A previous interpretation of the strontium-90 level in Felinfach milk samples during 1958 indicated that less than 35 per cent was derived from the reservoir of activity accumulated in the soil[7]. In 1960, with a larger soil reservoir and a much-reduced rate of fall-out, it might be expected that the levels in milk would be determined to a greater extent than before by the soil reservoir activity. This appears to be borne out by the fact that in 1960 the strontium-90 level in milk did not drop in the same degree as did the rate of fall-out (Fig. 1). In comparison, the trend of cæsium-137 levels in milk followed the fall-out trend more closely, indicating that in this case the contribution from the soil reservoir was less important. The demonstration (Table 1) that the cæsium-137/strontium-90 ratio of activities in the milk decreased progressively during 1958–60 is consistent with this interpretation. In the analysis which follows these various relationships are assessed more closely.

Attempts have been made previously[8–10] to establish quantitative relationships between the levels of activity in milk, on one hand, and the rate of deposition and the cumulative deposition, on the other, using equations of the type:

$$C = a.D_p + b.D_t \qquad (1)$$

where C represents an activity-level in milk, D_p an amount of activity deposited over unit area of ground during a short selected time period (rate of fall-out), D_t the accumulated total of activity deposited over unit area until any given time (cumulative fall-out), and a and b are proportionality factors. These attempts have been hampered by a lack of adequate data and in addition there has been an unfortunate tendency to use yearly average values for the C and D_p parameters. Since yearly average values for C incorporate winter values which reflect in large measure levels of contamination in stored fodder, the meaning of such correlations is often difficult to assess. In the present analysis, we restrict ourselves to consideration of levels of activity in samples which were produced during May–October periods, and we use monthly average values for strontium-90 (10^{-12} c.gm.$^{-1}$ calcium) and cæsium-137 (10^{-12} c.gm.$^{-1}$ potassium) activities in milk. Our values for activities in milk are listed in Table 2, together with estimated values for the monthly rates of deposition (mc. km.$^{-2}$ month^{-1}) and the cumulative totals (mc. km.$^{-2}$) at each month. The deposition figures have been computed from published information on the strontium-90 and cæsium-137 concentrations in rain-water collected at Milford Haven (50 miles south-west of the Felinfach area[11–13], and recently at Sutton in Surrey[14,15], together with the average of rainfall data from five stations located at different altitudes in the Felinfach area. The cumulative totals listed in Table 2 have been corrected to allow for the radioactive decay occurring in each monthly increment of fall-out between the time of deposition and the time at which the total is recorded. We

Table 3. COMPARISON OF COEFFICIENTS OF CORRELATION FOR VARIOUS REGRESSIONS BETWEEN RATE OF FALL-OUT (COMPUTED IN DIFFERENT WAYS) AND MILK ACTIVITY-LEVEL

Periods* used to compute rate of fall-out	(*Pn*)	P_0	P_1	P_2	P_0+P_1	$P_0+P_1+P_2$	$P_2+P_3+P_2$	$P_0+P_1+P_2+P_3$
Coefficients of correlation based on regression analyses made according to equation (2) (r_{12})	Strontium-90	0·73	0·91	0·75	0·94	0·95	0·94	0·91
	Cæsium-137	0·74	0·83	0·75	0·91	0·95	0·94	0·90

* P_0 corresponds to the month during which the milk was produced, P_1 to the month immediately preceding milk production, and so on for the other *Pn*. In the analyses which used combinations of fall-out from different months, the numbers taken to represent the rate of fall-out were the arithmetical averages of the sums of the deposits (mc. km.$^{-2}$) for the months involved.

now consider the information contained in Table 2, according to the methods of correlation analysis[16,17].

In considering the correlation of activity-levels in milk with the average of deposition figures over comparatively short periods of time, it is important to take into account factors which may introduce a lag correlation effect. Earlier reports, showing that strontium-89/strontium-90 ratios for both herbage[1] and milk[7] were smaller than those for rain-water collected during the same month, pointed to some degree of integration of recently deposited activity in the herbage, but in the absence of full knowledge of the role of the cumulative deposit it was not possible to express the extent of this effect in a simple fashion. In the present analysis we approach this problem by examining the degrees of correlation which hold between, on one hand, the activity-levels in milk (X_1) for eighteen open pasture feeding months (Table 2), and on the other, various combinations of the monthly amounts of deposition, which may correspond more or less to an effective rate of fall-out (X_2). These relationships are treated as simple linear regressions according to the equation:

$$X_1 = a + b_1 X_2 \qquad (2)$$

A preliminary examination of scatter diagrams for the two variables indicated the presence of a similar degree of positive correlation when the X_2 values were represented either by the deposition occurring during the month (P_0) in which the milk was produced, or during the previous month (P_1), or during the second previous month (P_2). When deposition values for months before P_2 were used, there was a significant and progressive decrease in the degree of correlation. This result applied equally to strontium-90 and cæsium-137 data. Various combinations of depositions from two or more months were now used to represent the X_2 values, and correlation coefficients were calculated in several important cases. These coefficients are presented in Table 3. It may be noted first that the degree of correlation obtained using the deposition of the month P_1 was higher than in all other cases for which single monthly depositions were used. Secondly, it may be noted that a significant increase in the degree of correlation occurred when certain combinations of monthly depositions were used, the maximum being obtained with depositions from P_0, P_1 and P_2 grouped together. The correlation result for this latter combination is illustrated in Fig. 2 for strontium-90 and in Fig. 3 for cæsium-137. It appears from the analysis that the rate-dependent component of activity in the milk is largely determined, both in the case of strontium-90 and cæsium-137, by the deposition in the month preceding milk production together with smaller contributions from deposition during the current and second previous months.

We now consider the extent to which the levels of activity for milk may be influenced by the reservoir of activity accumulated in the top of the soil. It appears that both strontium-90 (ref. 1) and cæsium-137 (ref. 18) may be largely held within the top few inches of the soil for comparatively long periods of time. A number of factors[4] may lead to a progressive reduction in the availability of this activity to the herbage, but at the present time the rate at which this process occurs is not well established. For the present purpose we assume that the effective soil reservoir is proportional to the cumulative deposit and we denote the levels at each month (Table 2) by X_3. The effective rate of fall-out (X_2) is taken to be the arithmetic average of the sum of the depositions during the months P_0, P_1 and P_2. Analysis of the data to include the second independent variable requires the use of a multiple regression equation which may take the linear form:

$$X_1 = a_{1\cdot23} + b_{12\cdot3}X_2 + b_{13\cdot2}X_3 \qquad (3)$$

Application of this regression equation to the data provides a basis for evaluating the proportionality factors appearing in equation (1):

$$(X_1 = C;\ X_2 = D_p;\ X_3 = D_l;\ b_{12\cdot3} = a;\ b_{13\cdot2} = b)$$

Restricting the regression plane to pass through the origin ($a_{1\cdot23} = 0$), we obtain the following results:

$$\text{for strontium-90: } C = 15.1\ D_p + 0{\cdot}28\ D_l \qquad (4)$$

with a standard error of estimate (S) = $1{\cdot}8 \times 10^{-13}$ c.gm.$^{-1}$ calcium and coefficient of multiple determination (R^2) = 0·85, and:

$$\text{for cæsium-137: } C = 84{\cdot}1\ D_p + 0{\cdot}36\ D_l \qquad (5)$$

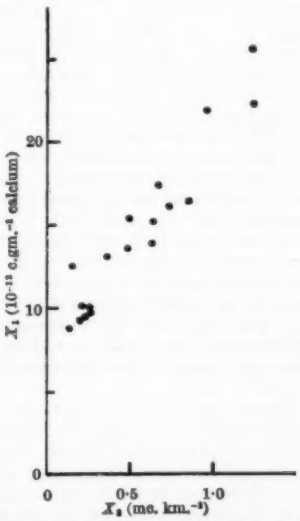

Fig. 2. Correlation chart of rate of strontium-90 fall-out (X_2), taken as average of sum of depositions during months P_0, P_1 and P_2, and monthly strontium-90 level in milk (X_1)

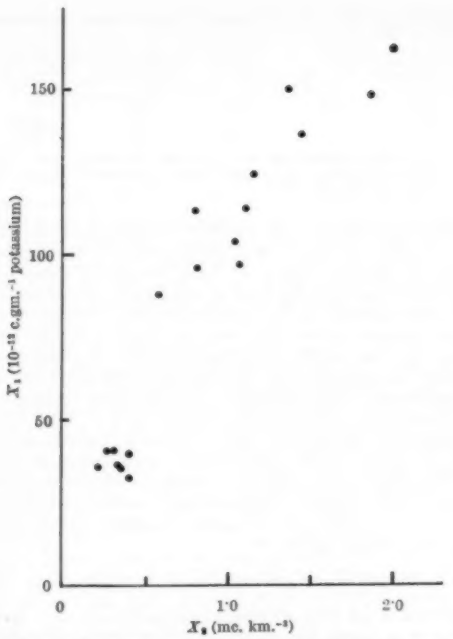

Fig. 3. Correlation chart of rate of cæsium-137 fall-out (X_2), taken as average of sum of depositions during months P_0, P_1 and P_2, and monthly cæsium-137 level in milk (X_1)

with $S = 16{\cdot}0 \times 10^{-12}$ c.gm.$^{-1}$ potassium and $R^2 = 0{\cdot}88$.

The values of the standard errors and the coefficients of determination show that equations (4) and (5) provide a fairly close description of the strontium-90 and cæsium-137 data, respectively, for the open-pasture periods of 1958–60. An application of equation (4) to earlier strontium-90 data also gives estimated levels close to the observed values ((1) October 1957, Felinfach area[6]—observed $9{\cdot}7 \times 10^{-12}$ c.gm.$^{-1}$ calcium, estimated 8·1, (2) May–August 1957, pastures at Carmarthen adjoining Felinfach area[10]—average value observed 7·0, estimated 7·8, (3) October 1956, Carmarthen[1]—observed 8·0, estimated 7·5). An evaluation of the rate and cumulative components of the equations suggests that during May–October 1960 the soil reservoir activity accounts for about 70 per cent of strontium-90 in the milk and about 35 per cent of cæsium-137. It may also be noted that the ratio of the proportionality factors in the equation for strontium-90 ($a/b = 54$) is markedly different from that for cæsium-137 ($a/b = 234$). A change in deposition-levels would not therefore be expected to have an equal effect on the level of strontium-90 in milk compared with that of cæsium-137. This provides an explanation of the changes observed in the cæsium 137/strontium-90 ratios for the milk samples produced during 1958–60 (Table 1).

The results of the present analysis apparently provide a useful guide to the degree of contamination which might be expected to arise in milk under a given set of deposition conditions, and at the same time indicate the extent to which this contamination might persist. The results are strictly applicable only to the Felinfach area, but, as noted at the outset, it appears likely that the corresponding relationships for other regions in Britain are broadly similar in magnitude to those shown in equations (4) and (5).

The trend in levels of activity of milk during 1961 will depend partly on the rate of transfer of activity from the stratospheric reservoir to the troposphere, and also on the extent to which direct additions of activity may be made to the troposphere during the new series of weapons tests that was begun in September 1961. Measurements of the concentration of strontium-90 in rain-water collected at our laboratory during the early part of 1961 give values which are about 1·5 times smaller than the corresponding values for 1960. These results, together with the rainfall data available at present, give a preliminary indication that the rate of fall-out during January–August, 1961, will be smaller than that for the corresponding period of the previous year. Consequently, the rate-dependent components of activity in the milk may also be expected to decline. An increase will occur in the cumulative deposition between mid-1960 and mid-1961 but the increment should be less than 5 per cent of the mid-1960 total. Further, this increase may be more than offset by depletion of the effective soil reservoir due to small changes in the distribution of activity within the top layer of the soil. This is suggested by the results reported by Squire[4] of experiments on plants grown in soils treated with strontium-90, where it was found that the availability of the activity to the plants decreased by about 25 per cent over a period of 3 years. The available evidence therefore indicates that a further decrease of activity-levels will be observed in milk produced during the summer and early autumn of 1961.

We thank the Milk Marketing Board for the supply and preparation of the milk samples, and also the Meteorological Office and the Welsh Plant Breeding Station (Aberystwyth) for information on rainfall. Valuable assistance in the chemical analyses was given by Mrs. A. Perry. We are indebted to the director of this Department, Prof. W. V. Mayneord, for his constant support and guidance.

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FISH IN NUTRITION

THE Fisheries Division of the U.N. Food and Agriculture Organization, in collaboration with the Divisions of Nutrition and of Animal Production and Health, were responsible for organizing a conference which, at the invitation of the Government of the United States, and with generous financial assistance from the Division of General Medical Sciences through the agency of the National Academy of Sciences, was held in the new conference auditorium of the Department of State, Washington, D.C., during September 19–27. Various North American commercial fish organizations also combined to make the gathering a success.

The conference, which was attended by more than three hundred official participants and various experts and industrialists from more than thirty countries, was opened by Dr. D. B. Finn, the director of the Fisheries Division, as representative of the Director-General, after an address of welcome by the Hon. Stewart L. Udall, Secretary of the Interior. There was also a message of greetings from President Kennedy.

The chairman of the conference was Mr. Donald L. McKernan, director of the Bureau of Commercial Fisheries of the Fish and Wildlife Service of the U.S. Department of the Interior, and five main topics were discussed, each under a distinguished chairman, with a rapporteur and secretary, covering exhaustively the various biological, chemical, technological, nutritional and economic aspects of fish and fish products. For each topic a number of review papers, thirty-four in all, had been invited from well-known authorities, supplemented by thirty-five selected original short research papers. These were all circulated beforehand, the latter as abstracts only, and contributors briefly introduced their papers. The edited proceedings, including the various recommendations and conclusions, are to be published by Arthur J. Heighway Publications, Ltd., 110 Fleet Street, London, by arrangement with the Food and Agriculture Organization, next year.

As it is impossible in the course of a short article to cover adequately such a broad and detailed agenda, a personal selection has had to be made of the various important issues that were discussed by the numerous experts present.

Productivity of the Seas

There was a clash of opinion among the marine biologists on the maximum harvest that could be expected from the seas. Some took the conservative view that knowing the quantity of solar energy that can be fixed by marine plants and making certain assumptions as to the number and efficiency of the conversion steps in the food chain, one cannot hope for much more than double, or at most treble, the present catch of slightly less than thirty million tons of marine fin fishes per annum. Others pointed out that landings from the oceans have already nearly doubled in the past ten years, and thought that there was reason for believing that, even using present methods of fishing, five or even ten times the present yield could be achieved and sustained.

At present commercial fishing operations are largely determined by market considerations. Many edible species, such as sharks, are often not brought home even when caught. A two million ton per annum fishery of anchovies used for fish meal has sprung up off the coast of Peru in less than a decade. Some American biologists believe that a similar potential exists off the coast of California, and that there are also untapped resources of saury, which would be despised as a food fish in the United States, although since the War it has been canned on a large scale in Japan. Improved methods of preventing decomposition, such as freezing at sea, is enabling Russian and Japanese ships, for example, to exploit fishing grounds all over the world.

Again, greatly improved methods of fishing can be envisaged. In spite of much effort on the part of the Food and Agriculture Organization and Governments, there is still a several hundred-fold difference between the highest and lowest productivity per fisherman. The suggestion was revived of a planned ploughing of the sea through the convectional effect of nuclear reactors, by means of which nutrient-rich ocean depths could be brought into the life-cycle of the upper layers. Finally, also looking well into the future, there is the prospect of fish farming on a scale as yet unimaginable, perhaps involving the intelligent utilization of the organic material at present prodigally wasted in sewage.

However, these distant vistas do not dispose of the fact that here and now in certain more easily accessible areas there is a decided falling off in yield due to uncontrolled fishing, with uneconomic results. The future yield in such cases is not determined only by Nature, but depends in large measure on the decisions of man himself. Man acts not only as a predator and disturber, but also as an intervener in natural systems, so that the fish stock of the future, in the words of Dr. G. L. Kesteven (Cronulla, New South Wales), "is likely to be so much more or less than it is now, as man makes it to be".

It therefore seems that, although there is no justification for pessimism, more work will be needed before the basis exists for even an approximate estimate of the potential yield of the oceans.

Unequal Distribution of Fish Supplies

Added to the uneven development of the present world fish catch is the inequality of its distribution. In this respect there is a general lack of realization of the implications of the tremendous gap that exists between the 'have' and 'have-not' nations.

Although the world dietary deficit in calories is relatively slight, so that probably relatively little actual starvation occurs, there is a general protein under-nourishment over much of the globe which greater consumption of fish could do much to remedy. Even where national statistics which are reliable indicate adequate average diets, deficiencies are known to exist in special regional or socio-economic groups. Children in the age group 1–4 years have special protein needs but often do not receive a proper share of the right foods. Kwashiorkor and marasmus are protein-deficiency conditions of infancy which cause many fatalities, particularly in Africa and South America. Women frequently pregnant and lactating equally require high animal protein intake, but all too often men get priority. Protein

under-nourishment, even where not fatal, is responsible for untold misery, debility, disease, and retarded growth and development.

Although fish accounts for only about 12 per cent of the total animal protein available, it nourishes far more than this percentage of the world population. For more than 1,000 million people it constitutes the chief source of animal protein. Milk and meat products, although bulking much larger, feed far fewer millions. Fish is the protein of the poor, nationally and globally.

Over North America and most of Western Europe the consumption of other animal products is so much greater than that of fish that the latter could practically disappear altogether from the diet without being missed. However, fish is equally nourishing for farm animals, the requirements of which have received much closer examination than those of human beings. The high and increasing output of meat products in the West is facilitated by converting about 5 million tons of edible fish annually into feeding meal for animals. Only a small proportion of this fish meal comes from recovery of industrial waste. The bulk is from whole, edible fish caught entirely for reduction, much of it in Africa and South America. 95 per cent of this trade goes to produce milk, bacon and eggs in the countries least requiring extra nutrition. For example, one third of the broiler production in the United States can be attributed to the sea.

One of the topics which received greatest attention at the conference was the part that the polyunsaturated fatty acids of fish oil could play in the prevention of obesity and coronary and cerebral thrombosis, associated with excessive meat consumption, which are now among the major killers in the 'over-developed' nations (as they were termed). It is a grim irony that a contributory factor to such 'over-nourishment' (itself a special form of malnutrition) is the fish protein concentrate drawn from the very continents where protein under-nourishment is so rife.

But quantities that are essentially only of marginal interest to the West represent the difference between health and disease for many millions of people on the verge of starvation. Thus, if the enormous fish production of Peru, at present exported as fish meal, were consumed in South America instead, it could raise the nutritional status of that continent to the level of that of Italy.

The inequitable distribution of fish protein resources is further flaunted in the 300,000 tons of raw fish used yearly to produce fur on the mink farms of the United States and Scandinavia for a form of apparel used not so much for the warmth as the adornment of the wealthy. To quote the report issued at the end of the conference: "It is clear that 400 million people cannot continue to get the overwhelming part of the world's animal protein, while 2,500 millions are neglected and deprived of this essential nutrient". The situation is made even more acute by the expected doubling of the world's population to 6,000 millions in the next 40–50 years.

Utilization of Fish

Great disparities also emerged in considering the efficiency of utilization of fish as food for man and beast.

There was a general consensus of opinion that fish is a first-class food, rich in high-quality protein, valuable lipids and most of the fat- and water-soluble vitamins and minerals. However, there are appreciable losses of nutrient in handling, processing and storage which, in extreme cases, can amount to a sheer wastage of as much as three-quarters of the substance of the fish before it is consumed. Wastage occurs in trimming raw fish, by which much of the edible flesh is left on the bone, to the benefit of fish meal again. Protein and water-soluble vitamins escape during salting, and both can suffer as much as 50 per cent loss of quality during severe drying processes. Over much of the world insects cause further loss, as much as 50 per cent being reported from Nigeria.

Furthermore, there is, even in well-developed countries, the imponderable consideration of the effect on fish consumption of loss of palatability on account of chronically careless handling of a very perishable commodity between catching and consumption.

Although there is much more that could be found out by further research, it is not lack of knowledge that is holding up technical advance so much as the lack of application of established knowledge and experience. In many cases the formulation and adoption of suitable standards of processing the product are seen as a means of providing the impetus for technical improvements.

There was a difference of opinion about the toxicity of oxidized fish oils, attributed to the extreme reactivity of hydroperoxides. Such oxidation, associated with many traditionally cured products, is also partly responsible for their characteristic flavour, which is often greatly relished in a cereal diet in circumstances where fish is regarded more as a condiment than as a source of protein.

Refrigeration and canning, which are serviceable stock methods of preservation under a wide variety of conditions, result in little or no loss nutritionally. There did not seem to be much hope that any of the newer methods of preservation, however satisfactory nutritionally, could be expected to assume such importance that proper care and control over established processes would soon be unnecessary. Vacuum freeze-drying of fish seemed unlikely to compete in quality with freezing in the West, or in cheapness with the traditional methods used elsewhere. The possibilities of sterilization by radiation, too, seem to have been greatly over-rated. On the other hand, the recent development of a large Japanese industry for manufacture of fish sausage was noted with interest, and thought to be a means of extending the outlet for fish in many other areas. Consideration would, however, have to be given to the advisability of encouraging the use of the chemical preservatives permitted in Japan.

Much discussion centred around the possibility of producing a tasteless fish flour that could be introduced particularly into the diets of young children in needy areas. The technical difficulties and achievements to date were reviewed and standards proposed for products of acceptable quality and nutritive properties. While some saw in fish flour "the basic fishery product of the future", others felt that much more could be done here and now to improve nutrition by means of the application of knowledge and experience already available for types of fishery product known to be generally acceptable.

Economic and Educational Problems

Without denying the need for immediate emergency schemes for using fish as nourishment, other animals are not necessarily competitors with man for the fish

supplies. A good case can be made out for the use of fish for animal feeding as part of a planned protein economy, although at present this is of more interest to the West than to the rest of the world. Much vegetable protein, in the form of grass, for example, is made available to us because cattle diets are supplemented with a small amount of fish meal. Although there is a loss of energy at each step in the food-cycle, protein may be converted with 50 per cent efficiency in ideal circumstances. Essential amino-acids such as lysine and methionine may be recovered at efficiency-levels up to 70 per cent in animal products such as hen's eggs. Ruminants, particularly, can make better use of fish products regarded as of low quality for other purposes.

However, it was admitted that animal feeding is a very opportunist operation, which makes use of whatever products and by-products are available at suitable prices. If the fish at present used for fish meal were to be upgraded to, say, fish flour, for human use, the industry would be forced once again to look for alternative sources of supply and would have to rely on industrial fish waste and such feeds as soya bean meal supplemented with synthetic amino-acids, although at somewhat higher cost.

In the world of commerce it is more profitable to make fish meal or raise mink than it is to make and distribute food for children of low-income groups. However, there are circumstances in which nations cannot afford to count the cost, and a concerted attack on freeing the world from hunger is surely a worthy cause. The Food and Agriculture Organization has, in fact, initiated such a campaign which it is the duty of the more fortunate nations to support to their utmost. However, even if economics were over-ridden in some such manner, there are technological and educational problems to tackle in getting fish consumed where it is needed. It is true that this is only one aspect of accelerating a general expansion in production and standard of living in underdeveloped countries. But more organized technical assistance needs to be given before many countries are in a position fully to help themselves. The Organization needs further support in making their self-help more effective. Training of personnel at all levels should have greater priority. Fishermen and others have to be taught to use better methods of catching and preservation. Consumers need education in the development of better dietary habits. Technical personnel, research workers and administrators all need training before programmes of fisheries expansion can be implemented.

Obviously this cannot be done overnight. However, it was felt by many at the conference that more effort should be put into education by providing expanded facilities for technicians to be trained to understand and utilize improved methods based on present knowledge and experience.

Finally, there was considerable support for the thesis that, although admitting that much of what is already known is not being made use of, increased support for fundamental research is essential for the most efficient utilization of fish in the future.

C. L. Cutting

THE INTERGOVERNMENTAL OCEANOGRAPHIC COMMISSION

THE oceans, which cover some 70 per cent of the surface of the Earth, are perhaps one of the greatest fields for scientific investigation. They contain unlimited areas of study in practically every scientific discipline and yet, measured with the yardstick of our knowledge of the Earth and the atmosphere, it must be admitted that we have to learn much more about the sea before we can hope to understand its vagaries and make full use of its resources.

We know enough about it to appreciate the complexity and vastness of the problems which confront us, and there can be no doubt that many of them can only be solved when scientists co-operate on an international scale. Although one is apprehensive in condoning the establishment of more international organizations labelled with forbidding combinations of the alphabet, there is no doubt that the establishment of the Intergovernmental Oceanographic Commission (I.O.C.), which held its first meeting in Paris during October 19–27, fulfils a long-felt need and promises better co-ordination in the future for marine science.

International organizations dealing with the science of the sea have existed for many years, but they have been either of a regional nature or confined to a limited scientific field. In 1957 the International Council of Scientific Unions foresaw the necessity for a wider scale of co-ordination of activity, both geographically and disciplinary, and it established a Special Committee on Oceanic Research (S.C.O.R.) to consider such problems. The activities of the Committee are, however, primarily scientific, and although, as one of its projects, it has initiated an international expedition to the Indian Ocean, it still remains a non-governmental organization solely dependent on co-operation with national academies or similar scientific organizations.

The Intergovernmental Oceanographic Commission, on the other hand, now makes possible a direct approach to participating governments and forms a very necessary link where projects involving considerable expenditure are contemplated. At its first meeting, Dr. Anton Brunn, of Denmark, a leading biological oceanographer, was elected chairman. Dr. William M. Cameron, director of oceanographic research for the Canadian Department of Mines and Technical Surveys, and Vice-Admiral V. A. Tchekourov, head of the Hydrographic Service at Leningrad, were elected as vice-chairmen. The Commission appointed as its secretary Dr. Warren Wooster, director of the Unesco Office of Oceanography, which will act as secretariat.

The initial task of the new body was to define its own responsibilities and the manner in which it would function, particularly in relation to existing organizations. The decisions reached show that it is prepared not only to receive the advice of existing inter-governmental and non-governmental organizations which at present advise Unesco on oceanographical matters, but also the hope was expressed that the Food and Agriculture Organization, the World Meteorological Organization and other specialized agencies of the United Nations would co-operate actively with the secretariat of the Commission.

XUM

Numerous proposals for joint international oceanographic programmes were made by member States and a list of these was selected for particular consideration. Many of the proposals amounted to regional surveys of interesting and important parts of the ocean, but it is also encouraging to note that some novel ideas were put forward. The proposal from the United States for a synoptic survey of the North Atlantic Ocean emphasizes a long-felt need in oceanography, which could only be accomplished by the ships of several nations working together. Some proposals by the U.S.S.R. included plans for studying the time changes in physical characteristics of the oceans, while others were aimed at the study of limited fields of currents which might be expected to yield more information about the mechanism of the processes occurring in the sea. Such fundamental studies are an essential corollary to survey work if any real understanding of water movements is ever to be reached.

An awareness of the difficulties facing large co-operative programmes was shown by a resolution submitted by the French delegation, who noted with concern the present shortage of experts in the marine sciences. This shortage could well delay the whole development of marine science for many years to come, and it was proposed that an urgent appeal should be made to Governments to create the necessary permanent posts and assured careers for young scientists and technicians who are attracted by the marine sciences.

Another barrier to advancement noted by one of the working groups set up by the Commission was the present lack of standardization and intercalibration of oceanographical methods and equipment. With a number of nations participating in co-operative programmes, the necessity for more standardization and intercalibration becomes more urgent and the Commission has requested the Special Committee on Oceanic Research to appoint working groups of scientists qualified in the specialized fields to examine the present methods and make recommendations about the appropriate steps necessary to make them universally effective.

In its planning for the International Indian Ocean Expedition, the Special Committee on Oceanic Research has already commenced this task. Scientists from different countries have already met together to intercalibrate methods of measuring primary organic production, nutrient chemical analysis and zooplankton sampling methods. Agreement has been reached on a standard plankton net for use on the Indian Ocean Expedition.

The question of the co-ordination of activities in the International Indian Ocean Expedition was also the subject of discussion, and it was felt that although this had been most satisfactorily and energetically carried out by the Special Committee on Oceanic Research and its co-ordinator, Mr. R. G. Snider, in New York, the responsibilities of that body were primarily scientific, and the co-ordination of the participating nations in such expeditions should more rightly lie with the Intergovernmental Oceanographic Commission and its secretariat. The question whether the latter should take over these activities for the Indian Ocean Expedition was considered by the Special Committee on Oceanic Research at its meeting in Monaco, following the meeting of the Commission. While appreciative of the principle, the Committee considered that it would be a slow and complicated task to divide and transfer the co-ordination, but a mechanism was set up to take advantage of the new possibilities.

A further subject of discussion was a proposal for an international oceanographic research and training vessel to be sponsored by Unesco. While sea-training is essential, opinion was very divided on the practicability of operating such a vessel. In view of existing facilities for international exchange of scientists between national vessels, it was felt that there is no really outstanding need for a jointly owned ship, but that the idea should be kept in mind for consideration at some future date.

To assist in the urgent question of accurate positioning at sea, the working groups recommended close co-operation with the International Hydrographic Bureau and the International Civil Aviation Organization to see what can be done to expedite the development and establishment of navigational systems which will meet the requirements of detailed oceanographical investigations (for example, an accuracy requirement of $\pm$ 0·25 nautical mile).

The intended use of unmanned equipment in the study of the sea also raises a problem of communication and telemetering information from remote parts of the ocean. Existing frequencies in radio communications are rapidly becoming absorbed and the bureau of the Commission has been authorized to specify requirements which can be put to the next meeting of the Administrative Radio Conference.

Perhaps the one outcome of the meeting of the new Commission of most immediate interest to practising oceanographers will be suggested improvements in disseminating information. Not only should these improve the exchange of data, but also publicize plans of projected cruises with information on the facilities available to visiting scientists. Such information will be circulated in a newsletter from the Unesco Office of Oceanography.

Although a good deal of the meeting of the Intergovernmental Oceanographic Commission was occupied with putting its own house in order, it is clear that action has been initiated in a number of urgent problems. The Commission is in a position to place the needs of marine science before the Governments of member countries and will no doubt adopt a balanced attitude to the various disciplines involved. In this respect, the biologists attending the meeting felt some degree of apprehension as their own subject, although extensively studied, is ill-co-ordinated at an international level. After an informal discussion, they concluded that to ensure that the Commission should receive the best scientific advice concerning biological phenomena in the sea, it would be of the greatest importance to set up a forum which can give the proper international backing to biological oceanography and they have undertaken, with the approval of the Special Committee on Oceanic Research, to work for the formation of an International Association of Biological Oceanography.

Towards the conclusion of the meeting of the Intergovernmental Oceanographic Commission, a session was devoted to the memory of one of the greatest oceanographers, Dr. Fritjof Nansen, on the occasion of the centenary of his birth on October 10, 1861. A most eloquent tribute was paid by Prof. A. D. Dobrovol'skiy, who described many of Nansen's achievements in oceanography and exploration. Dr. G. Böhnecke spoke of his personal memories of Nansen, and Prof. H. Mosby concluded the session by showing some of Nansen's own lantern slides.

Ronald I. Currie

CHEMISTRY IN THE SERVICE OF MEDICINE

THE second British Congress on the History of Medicine and Pharmacy, organized by the Faculty of History of the Worshipful Society of Apothecaries of London in co-operation with the Chemical Society and sponsored by Glaxo Laboratories, Ltd., was held in London during September 27–29.

Prof. D. McKie, who occupies the chair of the history and philosophy of science at University College, London, was president of the congress, which had as its theme "Chemistry in the Service of Medicine". The twelve papers given provided an authoritative historical review of the services which chemistry has rendered to the development of medicine from the sixteenth century to the present day.

The inaugural session was held at Apothecaries' Hall on September 28, when Dr. A. G. Debus (Harvard University and University of Chicago) opened the proceedings with an account of Paracelsian doctrine in English medicine up to 1660. Prior to that date chemical methods and terminology were widely used in England on practical and theoretical levels. Chemical remedies and analysis did not originate with Paracelsus but were closely associated with the work of the iatrochemists. The impact of chemistry can be discerned in Paracelsian views on the origin of matter and in premature attempts to explain life processes in chemical terms. Theory was less popular in England than practice, but the present close bond between chemistry and medicine can be traced to the Paracelsian and Helmontian iatrochemists of the sixteenth and seventeenth centuries.

Dr. F. W. Gibbs (Royal Institute of Chemistry) emphasized the importance of Herman Boerhaave (1668–1738), who impressed a new, scientific, pattern on medical theory and practice in which chemistry had a central position. His *Elementae Chemiae* (1732) is a classic, the first volume being largely physical in outlook while the second is devoted to physiological, biochemical and clinical applications. The characteristics of his method were described with the aid of examples taken from his work on heat, pretended alkaline cures for gout and the stone, fermentation processes, and body fluids.

Prof. D. McKie spoke on "Chemistry in the Service of Medicine, 1600–1800" and described some of the many text-books of medical chemistry which appeared in that period. Notable among these was the *Cours de Chymie* of Nicholas Lemery, which appeared in many editions between 1657 and 1757. Boerhaave's work had transformed chemistry into an academic science *per se* and rid it of its pharmaceutical bias. The next line of progress was exemplified by Van Helmont's work on 'gas', the experiments of Boyle on combustion and respiration, and the remarkable theories of Hooke and Mayow, the influence of which was deferred by the rise of the phlogiston theory. With Black's discovery of 'fixed air' as an 'air' chemically distinct from common air, and with similar studies by Cavendish, Priestley and Scheele, this problem approached solution with the work of Lavoisier, whose discovery of the compositions of air and water, together with his calorimetric studies on respiration, opened the way to physiology.

The afternoon session at the Royal College of Physicians, with Dr. Charles Newman in the chair, was opened by Prof. F. G. Young (Department of Biochemistry, University of Cambridge), who traced the rise of biochemistry between 1800 and 1900. The idea that the chemical processes in living materials were subject to laws different from those applicable to the inorganic world lingered until almost mid-century. By the time it was largely rejected, the term 'organic chemistry' began to lose its original connotations and to be applied to synthetic compounds of carbons whether or not such substances had originally been derived from living matter. The controversies about fermentation and decomposition between Liebig and others, who included Schwann and Pasteur, were gradually decided in terms of the existence of intracellular enzymes. Ehrlich's early investigation on intracellular utilization of oxygen emphasized the role of activation of oxygen in the tissues for purposes of cellular combustion. The reasons why British chemists so largely ignored the possibility of applying their science to biology in general, and to animal metabolism in particular, are obscure, but may be found in the love of household pets (with dislike of animal experiments), the dominance of mathematical studies in British scientific education, and the controversies which followed the publication of *The Origin of Species*.

Speaking on the early history of pharmacology, Prof. W. D. M. Paton (Department of Pharmacology, University of Oxford) began by comparing the knowledge of drugs at the beginning of the nineteenth century, as shown in J. A. Paris's *Pharmacologia*, with that in Binz's *Lectures in Pharmacology* at the end. The main difference lay only in the general scientific background; although some important new drugs had been found in the intervening period the real revolution in therapeutics had scarcely begun. There was some exemplary work on the analysis of drug action and in structure/action relationships, but there was no consistent impetus or drive. When, therefore, did experimental pharmacology really get under way and what period could be defined as its 'early history'? Prof. Paton sought to answer this by studying the rate of change in the pharmacopœias, by tracing the rise and fall of different types of preparation and the introduction of substances pure enough to inject, by following the development of new 'unnatural' chemical structures, and by charting the rate of establishment of fully fledged departments of experimental pharmacology. All this type of evidence indicated that pharmacology was only now reaching maturity and that the main contribution which it would make to medicine, as well as to general biological science, was still to come.

Dr. W. D. Foster, of Dudley Road Hospital, Birmingham, and formerly of St. Thomas's Hospital, London, spoke on chemical pathology, which began as an extension of two ancient diagnostic procedures: uroscopy and the inspection of blood shed at therapeutic phlebotomy. Between about 1680 and 1740, men such as T. Willis, J. Locke, R. Boyle and B. Langrish conceived that useful diagnostic information might be obtained by chemical analysis as well as inspection of blood and urine, but because of the limitations of chemistry obtained no useful results. A simpler approach to blood analysis in which the weight of fibrin, red cell mass, serum protein and water

was determined, enabled Andral, in the 1840's, to establish that disease might produce chemical changes in the blood. The first really useful diagnostic test in chemical pathology was for the detection of albuminuria and was introduced largely through the work of three British physicians, W. C. Wells, J. Blackall and R. Bright, in the early nineteenth century.

The modern development of chemical pathology began during the first decade of the twentieth century, and depended on the introduction of venepuncture to obtain adequate blood samples and the development of colorimetric analysis which was both sufficiently sensitive and simple enough for clinical purposes.

At the third session, held at the Royal College of Surgeons on Friday morning, the help given by chemistry to the elucidation of certain important medical problems was discussed. Sir Charles Dodds traced the growth of endocrinology and of organic chemistry, the former from Addison's great work on the suprarenal capsules (1855) and Claude Bernard's work on glycogen, followed by Oliver and Schafer's experiments with an extract of the suprarenals in 1895, the latter beginning with Wöhler in 1828 and taking a great leap forward to synthetic organic chemistry with the contributions of Kekulé, Cooper, Van't Hoff and Le Bel. The synthesis of adrenaline was the first milestone, the second was the synthesis of thyroxine by Barger and Harington (1926), following its isolation by Kendall (1916) and the clinical work on myxœdema by Murray in 1891. In a remarkable diagram Prof. Dodds showed all the chemical activity which has stemmed from Addison's paper in 1855. Among the substances isolated and characterized from the suprarenals, and yet others derived from these substances, are ascorbic acid, adrenaline and the sympathome drugs (for example, amphetamine), œstradiol and its analogue stilbœstrol, cortisol and the derivatives triamcinolone and prednisolone, aldosterone and its derivatives, progesterone and analogous compounds, testosterone and the anabolic steroids, and, finally, noradrenaline and the whole group of antihistaminic drugs which stem from it. The stimulus for it all came from medicine and biology, and particularly from endocrinology.

Chemistry and cancer was the theme of the paper by Prof. Alexander Haddow, who discussed the role of chemical substances in cancer pathology and therapeutics. In 1775 Percival Pott had written a classic account of cancer of the scrotum in chimney-sweeps which definitely incriminated soot as the causative agent. During the industrial revolution, coal-tar, mineral-oil and pitch were added to soot as materials capable of causing a great deal of industrial and environmental cancer, as exemplified in Joseph Bell's description of skin cancer among workers in the Scottish shale-oil industry. The term 'carcinogen' was first used in 1853 by Sir James Paget, who cited Lebert (1851) as proposing that a chemist must aid the pathologist in research on cancer. After many attempts, experimental cancer was produced artificially by coal-tar by Yagamura (1915) and during 1921–26 Block showed that cyclic hydrocarbon was the active carcinogen. At about the same time the work of Cook and Kennaway vindicated Lebert's forecast and provided, using artificial tars, virtual proof of the carcinogen hypothesis, which was further extended by the implication of 3 : 4 benzpyrene, certain biological alkylating agents and other substances. It was stressed that there was no antithesis between the study of viruses and chemical carcinogens, for chemistry was essential to both. Chemotherapy, which had begun with Lissauer and the use of arsenic in the treatment of leukæmia, was already developing rapidly, as shown by the use of stilbœstrol in breast cancer and of other useful products such as myleran.

Dr. T. F. Macrae, speaking on chemistry and nutrition, said that in the development of improved nutritional standards, especially in the past thirty years, chemistry has played a major part. Increases in crop yields have largely been due to synthetic fertilizers, adequate supplies of which have been ensured by the early discovery of nitrogen fixation. More recently further advances have accrued from the wide range of pesticides now available.

In the field of nutritional deficiencies the work of chemists on vitamins has been particularly beneficial. All the important vitamins are now available synthetically at costs far lower than from natural sources. Indeed, in advanced countries at least, vitamin deficiencies are no longer encountered. Similar developments are likely with the amino-acids, it now being a simple matter analytically to determine the adequacy or otherwise of a protein source. Already in animal nutrition it has in some instances proved more economic to provide part of the nutritional requirements in synthetic form.

There still remains, however, the problem of inadequate nutrition, especially of protein, in the under-developed countries. This is in part technological since, though we can identify the deficiency and, in chemical terms, propose the remedy, we still have to find sufficiently cheap means of providing this remedy in a form acceptable to the populations concerned.

In the final session, held at the Chemical Society's rooms with Sir Robert Robinson in the chair, Prof. D. Catcheside reviewed the relationship between chemistry and genetics, beginning with the rediscovery of Mendel's work in 1900 and the speculation about the nature of his 'elements'. Chemical knowledge of their action is more advanced than that of the genes themselves. It began with the study of the differences between phenotypes determined by single Mendelian differences, such as the flower pigments of snapdragons by Muriel Wheldale (Onslow). This showed that a gene controlled the onset of a specific chemical reaction. But the conception of a gene-enzyme-chemical reaction system was due to Garrod's work on alcaptonuria and other inborn errors of metabolism in man. He considered that the abnormal gene resulted in absence or inactivity of the enzyme that normally cleaves homogentisic acid, in turn giving rise to disordered metabolism and disease. The theory was revived by Beadle and Tatum, following work on the eye pigments of *Drosophila*, when they reversed the procedure by searching in *Neurospora* for gene mutations that influence known chemical reactions. This has led, with the aid of the analytical techniques of Pauling, Sanger and Ingram, to the demonstration of specific amino-acid substitutions in abnormal hæmoglobins, and, with the development of microbial genetics, to attempts to correlate genetical and chemical detail for several proteins in fungi, bacteria and bacteriophage. As regards the genes themselves, geneticists in the first place showed that they were in the chromosomes, but direct implication of deoxyribonucleic acid, knowledge of the chemistry of which is a recent event due to Wilkins, Watson and Crick, resulted from microbial genetics. First, the demonstration that deoxyribonucleic acid is the sole substance concerned in transformation of *Pneumo-*

coccus (Avery *et al.*), and then that it is the vehicle of heredity in bacteriophage (Hershey *et al.*). More recently, attention has been directed to the coding problem and to the mechanism of transfer of specific information from deoxyribonucleic acid to protein.

A survey of progress "from antisepsis to antibiotics" was offered by Dr. W. A. R. Thomson, who said that although antiseptics had a long history, the antiseptic principle was first established by Lister in 1867, an event which led to a long and successful search for bactericidal drugs. Landmarks in that search were Ehrlich's '606', demonstrating the possibility of treatment by specific drugs, the introduction of the sulphonamides and, third, of the antibiotics. The usefulness of the sulphonamides had not yet been fully exploited and 'the antibiotic rush' of the fifties may in retrospect be seen as fundamentally unscientific. The isolation of 6-aminopenicillanic acid in 1959 marked the opening of a new and genuinely scientific era in antibiotic research. Dr. Thomson concluded with a word of caution; indiscriminate use of antibiotics had upset the balance of Nature and "the mass-murder of micro-organisms" was not necessarily a good thing. The careful use of antibiotics combined with immunotherapy offered a wiser course for the future.

Dr. F. L. Rose took one of the important developments of the same period for closer investigation in his account of the origin and rise of the synthetic drugs. With one or two exceptions, he said, all the medicaments available until the mid-nineteenth century were natural products. At that point in time, the coincidental discovery of the first synthetic dyestuffs and of micro-organismal origin of infectious diseases soon brought about a rapid change. The former because it caused an almost explosive growth of synthetic organic chemistry in a way which invited its direction into biological pathways, and the latter because it provided the motive and means for examining the effect on disease of the compounds poured out by the chemists. Thus the evolution of the modern antimalarial agents can be traced back directly to the observed staining of the malaria parasite by methylene blue. Methylene blue is a derivative of phenothiazine, itself widely used as an anthelmintic, and from which stem some of the most effective tranquillizing agents. Similarly, the modern trypanocides owe their beginning to the feeble therapeutic action shown by the early direct cotton dyestuffs, and the medicinal properties, initially antiseptic but later trypanocidal, found in the first azine and cyanine dyestuffs. Likewise, the appearance of the first azosulphonamide drug coincided with interest in the use of the sulphonamide group as a component of azo dyestuffs. These drugs were additionally important in that their technical development directed attention to the sulphones, now widely used in leprosy, while minor side effects were exploited to produce highly effective diuretic and hyperglycæmic agents.

A dinner was held in Apothecaries' Hall when the guest of honour was Sir Howard Florey, and the president of the Chemical Society, Sir Alexander Todd, gave the after-dinner address. He discussed the long-standing relationship between chemistry and medicine and stressed that the indebtedness was mutual, for medicine and its needs had provided a very great impetus to the growth of chemical research. He found in the title of the congress a reflexion of the orthodox separation of the basic sciences, a situation which was probably incapable of fruitful development. Many important problems required workers with many kinds of scientific skill, and although research institutes may provide this kind of team, research was still best undertaken by the universities. The barriers between departments must be broken down to enable the constant influx of young minds to cross the barriers between the sciences.

The papers given at the congress are to be published in volume form. It has already been decided that the meeting next year will be held in London during September 27–28, and will have as its theme "The History of British Hospitals". F. N. L. POYNTER

THE AUSTRALIAN NATIONAL RADIO ASTRONOMY OBSERVATORY

AUSTRALIA made an early start in radio astronomy at the end of the Second World War, and has remained ever since among the leading countries in this rapidly advancing field. Australian radio astronomers now possess a radio telescope which is the foremost of its type in the world at the present time—a 210-ft. steerable paraboloid, with a surface accuracy of a few millimetres.

The new telescope, which is located at Parkes, New South Wales, 230 miles west of Sydney, was commissioned on October 31 by the Governor-General of the Commonwealth of Australia, His Excellency the Right Hon. Viscount De L'Isle, and it will be operated by the Division of Radiophysics of the Australian Commonwealth Scientific and Industrial Research Organization. The telescope is the first instrument of the Australian National Radio Astronomy Observatory, which was inaugurated at the same time.

The plan for a large steerable paraboloid originated seven years ago, when the first specifications were drawn up by Dr. E. G. Bowen, Chief of the Organization's Division of Radiophysics. His vision and enthusiasm have been largely responsible for the successful completion of the project.

A large donation from the Carnegie Corporation (250,000 dollars) enabled the design work to start, and other generous donations were afterwards received from the Rockefeller Foundation (357,000 dollars), and from a number of private donors in Australia. These sums were matched on a pound-for-pound basis by the Australian Commonwealth Government. The total cost of the project has been about £800,000 (Australian) equivalent to £640,000 (sterling).

The novel design was produced by Freeman, Fox and Partners, consulting engineers, of London, with Mr. Gilbert Roberts in charge of the responsible group. The construction was carried out by Maschinenfabrik Augsburg-Nürnberg, A.G., of West Germany, which submitted the best tender, both in price and delivery time, and was awarded the prime contract. The principal sub-contractors were Askania Werke,

of West Berlin, Associated Electrical Industries, Ltd., of Manchester, and Concrete Constructions Pty. Ltd., of Sydney.

The telescope is mounted in azimuth-altitude style, on a single concrete tower, which also contains the telescope controls and the radio receivers. The whole structure rotates in azimuth on a circular track which is set into the top of the concrete tower; a range of 450° is available, limited only by the cable-twisting requirements. The altitude motion, between zenith angle 0° and 60°, is obtained through a large rack gear. A wide range of speeds is available in each co-ordinate.

A unique feature of the instrument is the control system for driving the telescope in equatorial co-ordinates. The heart of this system is a small 'master equatorial' unit, which is mounted at the intersection of the altitude and azimuth axes, on a special concrete support, completely isolated from the rest of the structure. This unit controls the motion of the telescope through a servo system which contains an optical link.

The 'dish' is supported by a structure of 30 radial trusses, which carry a system of ring girders and two sets of intersecting spiral members. The reflecting surface consists of wire mesh, with wires 1 cm. apart, except for the inner 54 ft., which is covered with steel plates. The inner solid section was adjusted to fine limits during construction; but the individual panels of the mesh surface carry screw adjustments, which can be reset at any stage.

The aerial feeds and pre-amplifiers are supported by a large cabin, which is mounted on a tripod above the reflector. The cabin is large enough to carry test equipment and observing personnel during special tests, though normal operations will of course be controlled from below. There are a lift in one tripod leg and ladders in the other two, for access to the focus cabin.

The telescope is now structurally complete, and is at present undergoing a rigorous series of tests, through theodolite surveys of the shape of the dish and radio observations of cosmic sources. Some progress performance figures are now available, and very satisfactory results are being obtained.

The original specification called for a surface accuracy of ± 9 mm. With the dish looking vertically, most of the adjustment points on the mesh surface are within 2 mm. of the required parabolic shape—with additional deviations of the same order inside the individual panels.

Some early figures have been obtained for the radio performance at wave-lengths of 21 and 10 cm. At 21 cm., the beam-width is $13\frac{1}{2}$ min. of arc or less, and the side-lobe level is in the region of -25 db. At 10 cm., the first measurement gave a beam-width of $7\frac{1}{2}$ min., and the side-lobe level is again very low. Moreover, these figures were obtained with the dish 40°–60° from the zenith, where any distortions would be at their maximum.

On the opening day, there was a widely representative gathering of nearly 500 guests at the Observatory, many of whom had come from Sydney, Canberra and Melbourne in a special airlift. Before the ceremony, the visitors inspected the structure, and then were shown a large photographic and descriptive display which had been specially set up for the occasion.

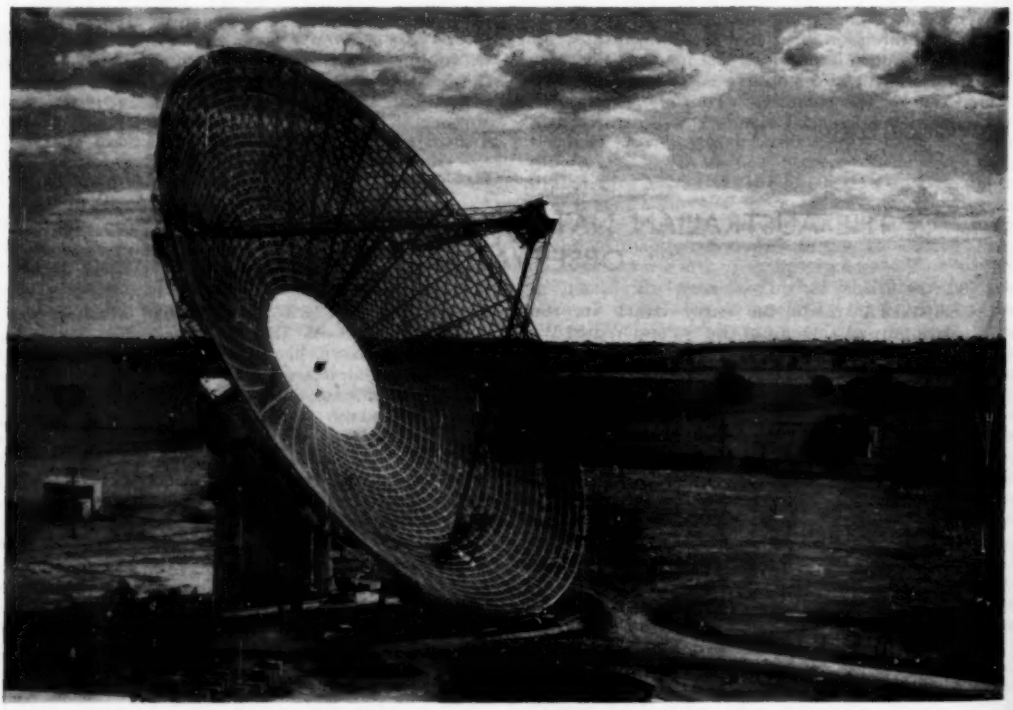

Fig. 1. The completed telescope, tilted down to its lowest position, 60° from the zenith

After being welcomed by Dr. F. W. G. White, Chairman of the Commonwealth Scientific and Industrial Research Organization, the Governor-General began by reading a message from H.R.H. The Duke of Edinburgh:

"The development of radio astronomy has been one of the great events of modern Science, and the scientists of C.S.I.R.O. have played a leading part in this important work. Their studies of the Sun and the exploration of extraterrestrial space to the remotest parts of the universe have gained the admiration of astronomers everywhere and have brought great credit to Australia.

"To-day the radio astronomers of Australia are to be rewarded by the inauguration of a magnificent radio telescope which will enable them to penetrate still further the secrets of the cosmos. This occasion marks the culmination of much planning and hard work and I send you my best wishes for the success of your investigations with this new instrument."

His Excellency then discussed the way in which realization of this "Giant Radio Telescope" had come about as the result of a happy conjunction in Australia of several distinct, but all of them propitious, circumstances. "Thus conceived and encouraged, the enterprise has leapt forward at a pace which I found both admirable and refreshing." He closed in whimsical mood with a quatrain which "seemed to float into my mind from an extra-planetary source":

"Twinkle, twinkle radio star.
We can't see you: you're too far.
Now we hear from outer space
How you run your cosmic race."

The next speakers were Dr. The Hon. D. A. Cameron, the Minister-in-Charge of the Commonwealth Scientific and Industrial Research Organization, and The Right Hon. Lord Casey, who was Minister-in-Charge of the Organization during the early critical stages of the project, and has continued his connexion with it as chairman of the Radio Astronomy Trust.

At this stage the Governor-General performed the commissioning ceremony, and a large Australian flag was broken out above the focus cabin, the highest point of the structure. Unfortunately, wind gusts of more than 50 m.p.h. interfered with a plan to tilt the dish down towards the assembled crowd. The area is normally very calm, which in fact was one of the reasons for its choice; but on this ceremonial occasion the weather was unkind, with wind-speeds at their highest level for several years.

Dr. Bowen closed the proceedings by outlining some of the plans for the future use of the instrument. A number of important observational programmes are ready to start as soon as the present period of extensive tests is completed. These include work on the identification of extragalactic sources, and studies of galactic structure, the Magellanic Clouds, bright hydrogen regions, and the planet Jupiter.

The new instrument will make a powerful addition to the facilities available to the Australian radio astronomers; but it is also a notable gain for the astronomical world at large. To quote Dr. Bowen's concluding remarks: "When the first urgent tasks of the next few months have been performed, it will be thrown open to astronomers from every part of the world with worthwhile problems to attack. We pledge ourselves to work in close collaboration with our fellow scientists from any country and will make this Observatory a rallying point for equipment and information, for techniques and intellects which, together, will lead to further great advances in the science of astronomy".

OBITUARIES

Prof. Robert Boutflour, C.B.E.

PROF. ROBERT BOUTFLOUR, who had resigned as principal of the Royal Agricultural College in 1958, died on October 27 in Cirencester at the age of seventy-one. He was educated at Barnard Castle School and Armstrong College—now King's College, Newcastle upon Tyne.

Boutflour's first appointment was lecturer in agriculture with the Lancashire County Council; this was followed by a similar post in the University of Leeds, and in 1921 he was appointed agricultural officer for the County of Lindsey. In 1922 he was made chief agricultural officer to the Wiltshire County Council, where he remained until 1926, when he was appointed the first and last national director of dairy husbandry.

He is best known for his work on the breeding and management of dairy cattle. This he started in Wiltshire, where it was realized that he was a man of sound progressive ideas, and in fact, many years ahead of his time, which is now proved inasmuch as his ideas are now accepted in general practice. His fame extended abroad as well as at home, and he is well known to many farmers of the territories he visited in the United States, Canada, Kenya and Australia.

While national director of dairy husbandry, he was attached to the Harper Adams Agricultural College, where he established his famous Friesian herd of dairy cattle. This enterprise proved to be an outstanding success and enabled him to put into practice those points of management that he had so strenuously advocated to farmers throughout the United Kingdom.

Boutflour was an impressive author and wrote many works on farm management in general, and the subject of dairy cattle in particular. While his success as a scientist was largely due to his keen powers of observation, coupled with patient and inspired reasoning, his ability to impress audiences or individuals was the result of his enthusiasm, alert mind and logical arguments coupled with a quick wit. In character and speech he was always most forthright, but his caustic remarks were usually offset with a characteristic twinkle in his eyes.

His appointment as principal of the Royal Agricultural College in 1931 gave him the necessary scope for his ability in organization and administration, and particularly to associate with young people, to whom he was always willing to give advice and encouragement. When he retired from the principalship in 1958 he had the satisfaction of knowing that during his term of office he had increased the number of students from 48 to more than 450.

During the Second World War, as executive officer for food production in Gloucestershire, he adopted many revolutionary methods to increase the output of food. One of his most daring ventures was to

plough up the Slimbridge pastures adjoining the River Severn, which for centuries had been the habitat of wild geese. His critics said that with this terrific fertility no cereal crop would 'stand', but once again he proved them wrong. He was created C.B.E. in 1952, and in 1955 he received the Sir Thomas Baxter Prize and Gold Medal for outstanding work in dairy husbandry.

However, it is by his personal character that so many will remember him, and among his personal characteristics was a 'human' appreciation of his fellow men and the possession in a very large measure of that great virtue—tolerance. His wife, who survives him, was the greatest help to him throughout his varied career, by her companionship and understanding. The passing of this colourful character has created a void which will be difficult, if not impossible, to fill, and to the older generation who knew him—and there are so many—Bob Boutflour will always remain in their memory with affection as a generous, likeable and very 'human' person of outstanding ability; while the younger generation will reap the benefit of his scientific work, and the valuable contribution he made to British and, in fact, world agriculture. W. T. Price

Dr. A. E. Western

Alfred Edward Western, who died on October 11 at the age of eighty-eight, was one of the few who have been able to carry on mathematical research while fully engaged in another profession: in his case, that of a solicitor. He was educated at Marlborough and at Trinity College, Cambridge, which he entered with an open scholarship in 1892. He was seventh Wrangler in the Mathematical Tripos in 1895; the year in which Bromwich was Senior Wrangler and E. T. Whittaker second Wrangler.

Dr. Western published several memoirs on the theory of algebraic numbers, and one on the theory of groups, during the years 1898–1911, and showed himself to be thoroughly at home in a subject which was then little cultivated in Britain. His standing as a mathematician was recognized by the award of the degree of Sc.D. by the University of Cambridge in 1910. He gradually became more interested in questions of numerical computation, and published several notes giving the numerical evidence which he had found for various conjectured formulæ of Hardy and Littlewood relating to the distribution of primes, to the occurrence of primes of the form $n^2 + 1$, and to Waring's problem.

During the last years of his life he was engaged (jointly with Dr. J. C. P. Miller) in completing a table projected by the late Lieut.-Colonel A. J. C. Cunningham, with whom he had formerly collaborated. This table, which is essentially a continuation of Jacobi's *Canon Arithmeticus*, is shortly to be published with the help of a bequest made by Cunningham to the London Mathematical Society for the purpose.

Dr. Western was closely associated with the London Mathematical Society over a long period, and rendered great service to it. He became a member of the Council in 1900, and treasurer in 1913 in succession to Sir Joseph Larmor. He retired from this office in 1950, and many members will recall the delightful speech he made at a dinner held at that time.

As a solicitor, Dr. Western was a pioneer in initiating 'legal aid', and it was largely from his work that the legal aid scheme used in courts in Britain developed.

He will be remembered by many as a man of great kindliness and of transparent sincerity and honesty of purpose. H. Davenport

NEWS and VIEWS

Geology at Trinity College, Dublin: Dr. R. G. S. Hudson, F.R.S.

The chair of geology and mineralogy at Trinity College, Dublin, vacated by Prof. W. D. Gill on his appointment to the chair of oil technology at the Imperial College of Science and Technology, London (see *Nature*, **191**, 651; 1961), has been filled by the appointment of Dr. R. G. S. Hudson, Iveagh Research Fellow in geology at Trinity. Dr. Hudson, who studied geology at University College, London, under the late Prof. E. J. Garwood, joined the staff of the Geological Department at Leeds in 1922, became professor of geology in 1939 and during 1940–45 acted as consulting geologist to various companies engaged in the search for oil in Great Britain. During these years he published mainly on the Dinantian and Namurian of the north of England, demonstrating the rhythmic succession of the Yoredale Series, the stratigraphical relationship of the limestone of the Craven Reef Belt, the pre-Namurian age of the Mid-Craven Fault, and helped in mapping a large part of the Craven Lowlands. He also worked with W. S. Bisat on the goniatite faunas of the Namurian and, in particular, detailed the goniatite succession in the Alport Boring in Derbyshire. During 1946–58 he was a senior research geologist and palæontologist with the Iraq Petroleum Company, leading field exploration parties in Kurdistan, Iraq (1949, 1950) and in the Oman Mountains of Arabia (1951, 1952), and publishing on the stratigraphy and faunas of these areas. Much of the present knowledge of the Middle East Mesozoic faunas, in particular the stromatoporoids and hydrozoa, is due to his work. He has received the Foulerton Award of the Geologists' Association and the Wollaston Award and Murchison Medal of the Geological Society. He has also been president of the Yorkshire and the Liverpool Geological Societies and the Palæontological Association, and a vice-president of the Geological Society. In 1959 he was made an honorary member of the Société Belge de Géologie et Paléontologie, and in 1961 he was elected to the Royal Society.

The British Nuclear Energy Society

On January 1, 1962, the British Nuclear Energy Society will be established in succession to the British Nuclear Energy Conference. The successful use of nuclear energy demands the application of many divisions of science and technology and a constant interchange of knowledge and experience between those trained primarily in these divisions. It was to provide a channel for this interchange that in 1955

the British Nuclear Energy Conference was established by the Institution of Civil Engineers, the Institution of Mechanical Engineers, the Institution of Electrical Engineers, the Institute of Physics and the Institution of Chemical Engineers, which were joined later by the Iron and Steel Institute, the Institute of Metals, the Institute of Fuel and the Joint Panel on Nuclear Marine Propulsion. The Conference has sought to fill its role principally in three ways: (1) by publishing under one cover, in the *Journal*, all the more important papers on nuclear energy presented to the constituent societies; (2) by keeping members of the individual societies informed of those meetings of the other societies that are devoted to aspects of nuclear energy and making such meetings open to them; (3) by sponsoring meetings on nuclear energy organized either for the Conference by the appropriate societies or, where the subject involves the application of several disciplines, by the Conference itself.

The time has now come to provide a central forum for the discussion of nuclear energy, and it is for this purpose that the British Nuclear Energy Society has been established. The Institution of Civil Engineers has made its premises in Great George Street, Westminster, available as a meeting place, and a regular programme of papers, lectures, symposia and informal discussions is being planned. The Society will (like the Conference) publish a quarterly *Journal* which in addition to reprinting a wide range of important papers will also contain original papers and discussion on them, information and authoritative scientific and technological comment on developments in nuclear energy throughout the world, and reports on the proceedings of meetings of the Society. The first issue of this *Journal* will be in January 1962.

Membership of the new Society will be open to members of all classes of the constituent societies of the British Nuclear Energy Conference and to all who satisfy the Board that they are (to quote the Society's constitution) "actively engaged in the professional, scientific, or technical aspects of the application of nuclear energy and ancillary subjects". Further information can be obtained from the Secretary, British Nuclear Energy Society, Institution of Civil Engineers, Great George Street, London, S.W.1.

Changes in Times of International Time Signal

For more than one hundred years, the Royal Greenwich Observatory has been responsible for providing exact time signals for a wide variety of users both in Britain and abroad. In recent years this service has become increasingly important in various fields of scientific research where extreme accuracy is essential. In order to provide the various users with more frequent opportunities for checking the time, the present twice-daily transmissions from Rugby were increased to four as from December 1. This will mean that the transmissions on the low frequency of 16 kc./s. now radiated at 10 a.m. and 6 p.m. will be superseded by signals at 3 a.m., 9 a.m., 3 p.m. and 9 p.m. There will, however, be no change in the form of the signals. To ensure a world-wide coverage, the 10 a.m. and 6 p.m. broadcasts have also been transmitted on short wave. This service will continue after December 1, but the times will be changed to 9 a.m. and 9 p.m. As long ago as 1833, the Royal Greenwich Observatory provided hourly time signals for the operation of 'time balls', which were devices consisting of a large ball secured to the top of a mast and released by a special catch at a precise time. One such ball is still in use in the grounds of the old Observatory at Greenwich. The hourly transmissions continued for many years, but this service has now diminished to almost negligible proportions and, in fact, the only user is the G.P.O. for the Talking Clock (*TIM*) which has been in operation since 1936. The reason for the falling-off of the requirement for the hourly Greenwich transmissions was the inauguration in 1924 of the B.B.C.'s Greenwich time signal, which is claimed to be as accurate as possible.

The Federal Council for Science and Technology

According to *Scientific Information Notes* (3, No. 5, October–November 1961), the U.S. Federal Council for Science and Technology will in future, to help scientific periodicals meet the cost of publishing research results, pay, as a necessary part of research costs under Federal grants and contracts, page charges for publication of research results. The research papers concerned must report research work supported by the Government and the charges must be levied impartially on all research papers published by the periodical. Payment of such charges must not be a condition for acceptance of manuscripts by the periodical, and the periodicals must not be operated for profit. The new policy was stimulated by the National Science Foundation. The National Science Foundation has also placed a contract for computer-aided research with the Ramo–Wooldridge Corporation, Canogen Park, California, to further the investigation of new techniques for processing language data. Under the contract, 300,000 words of Russian text, representing five fields of study, will be used for dictionary compilation. Concurrently, the translation programme will be used for mechanical translation research in which the translation will be automatically compared on the computer with the results of a previous revision of the dictionary. Part of the investigation will be to consider the introduction of modifications and supplements into the programme. At the Institute for Scientific Information, Philadelphia, molecular formulæ of several hundred randomly selected chemicals have been calculated on an electronic computer, using a newly constructed recognition grammar of chemical nomenclature, and the new grammar enables the computer to understand the chemical names fed to it by a typist. The linguistic analyses indicate that the procedure can be used manually by chemists in training clerks, librarians and indexers to calculate a molecular formula from a chemical name, and the new computer technique is being incorporated into the preparation of *Index Chemicus*.

National Central Library

The forty-fifth annual report of the Executive Committee of the National Central Library (Pp. 24. London: National Central Library, 1961) covers the period March 1, 1960–March 31, 1961, in which, on an average, applications for loans increased by 4·6 per cent each month over the corresponding month in the previous year, while the percentage dealt with successfully increased from 72·22 to 74·13. Volumes added to the general stock by purchase rose from 1,129 to 1,952, largely in foreign languages, and nearly 18 per cent of requests were met directly by loans from the Library's own stock, compared with 16 per cent the previous year. Satisfactory progress is reported in the first stage of the project for dealing with the arrears of cataloguing, but further funds are

necessary for dealing with the later stages of the project. The Library's British National Book Centre has now extended its service to include foreign libraries, and some 19,977 specially requested books and periodical volumes and parts were allocated to 126 foreign libraries, while in return 3,987 offers of foreign material were made by the foreign libraries benefiting. Of the total issues of 90,612 volumes, 16,555 were to university libraries, 10,255 to special outlier libraries, 2,653 to Government departments, 3,414 to industrial and research organizations, and 4,520 to overseas libraries. The university libraries lent 29,826 books, compared with 28,611 in the previous year. The Library itself supplied 641 microfilms and 326 photostat copies, and of these 491 microfilms and 187 photostat copies came from co-operating libraries in the United Kingdom. Foreign libraries supplied 732 microfilms and photostat copies.

The Institute of Personnel Management

The annual report of the Council of the Institute of Personnel Management for 1960–61 records an increase of 441 in membership to 4,938, and the Information Service is now handling about double the number of inquiries in 1959 (Pp. 31. London: Institute of Personnel Management, 1961). During the year, views on the proposed Government Bill on Health, Welfare and Safety in Shops, Offices and Railway Premises were submitted by the Institute to the Ministry of Labour. A paper by A. B. Cherns on priorities for research in the social services in industry was published in the Institute's journal, and during the year a sub-committee on research was formed which convened a meeting of representatives of research bodies in this field to advise it, at which the proposal that the Institute should develop its research role was welcomed. Two important new broadsheets have been published, one by H. E. Roff and T. E. Watson, dealing with job analysis, and the other by J. R. Armstrong on supervisory training, and an address by Sir Ben Lockspeiser, "Man and his Machines, Automation and Computation—their Origins and Effects", given at the 1960 National Conference, has been published as an occasional paper. Particulars of this conference and of the regional conferences held during the year are also given in the report.

Science and Public Health

The annual report for 1960 of the scientific adviser, Dr. S. G. Burgess, of the Scientific Branch, Public Health Department, London County Council, at present organized in eight sections, records that 42,090 samples were examined during the year (Pp. 60. London: London County Council, 1961. 1*s*. 3*d*.). Some 90 per cent of the increase of 6,800 samples is due to the analysis of air samples necessitated by safety precautions against the leakage of inflammable sludge gas at the Northern Outfall Works. During the year the resources of the Board were made available to the Metropolitan borough councils, and investigation of noise, and nuisance arising from noise, in a large area of London was commenced in collaboration with the Department of Scientific and Industrial Research. A light-weight concrete based on an expanded aggregate was used for the construction of a thirteen-storey block of flats, and the Scientific Branch co-operated with the architect in solving many problems associated with the design. The practicability of laying hot asphalt over an expanded polystyrene roof insulation was also investigated, a new washing technique developed which offers considerable savings in laundry processes, while other special investigations were concerned with methane gas detectors, impurities in sludge gas and deposits in sludge gas lines and flame traps. New laboratories were completed at the Northern Outfall Works and a new Building Materials Laboratory at County Hall, London.

Corrosion Science

The first quarterly issue of a new international journal devoted to the science of corrosion and protection of metals and entitled *Corrosion Science* made its appearance in August (1, No. 1. An International Journal of the Science of Corrosion and Protection of Metals. Pp. 76. (London and New York: Pergamon Press, 1961. Annual subscription: (*A*) for libraries, Government establishments and research institutions, 140*s*., 20 dollars; (*B*) for individuals who write directly to the publishers certifying that the journal is for their personal use, 70*s*., 10 dollars). The journal is published by Pergamon Press under the guidance of an executive editorial board consisting of C. Edeleanu, J. G. Hines, T. P. Hoar and L. L. Shrier of Great Britain, M. Pourbaix of Belgium and M. J. Pryor of the United States. The standard of reproduction is of the usual high quality associated with Pergamon Press. The editorial notice mentions, among other things, that papers on corrosion are at present published in many diverse chemical, metallurgy and engineering journals, and expresses the hope that the new journal will help to concentrate this information. That there will be an adequate flow of papers of appropriate quality to the new journal seems assured, in view of the continually increasing amount of work in this field.

The first issue of a new journal always presents some difficulties for both publisher and reader, for the former in deciding how to obtain appropriate papers, and for the reader in deciding whether the first issue is likely to be representative of the eventual standing of the journal. In the present case there are five papers occupying 75 pages, with sufficient breadth of content to offer something of interest to the majority of corrosion scientists. The composition of the executive editorial board is such that the journal should establish itself as of high repute and with a continuing breadth of appeal.

The Australian Museum, Sydney

The September issue of the *Australian Museum Magazine* (13, No. 11; September 15, 1961) contains illustrated articles on the boomerang, insect migration, vampire bats—true and false—the piranha and meteorites. It is recorded that Mr. F. A. McNeill retired from the curatorship of the Department of Crustacea and Coelenterates after 47 years service. He was a specialist on Australian Crustacea. An exhibit on whales and whaling has been completed. It contains models of fifteen different species, of whaling ships and of whales being flensed on deck. Maps show the areas in the Antarctic in which whaling is carried out, the location of shore stations, and the areas in which whales are protected.

Automatic Data Processing

A publication issued by the Department of Scientific and Industrial Research (*Automatic Data Processing*. By Derek Wragge Morley. Pp. iv+76+8 plates. London: H.M.S.O., 1961. 6*s*. net) seeks to provide guidance to the public about available tech-

niques and machines in this field. The booklet is well produced, but the journalistic style and lack of critical appreciation of the history and implications of the subject will make it considerably less valuable than might otherwise have been the case. A particular shortcoming is the complete absence of any reference to more authoritative literature—the reader is directed to various manufacturers for further information. While this may be suitable for intending purchasers, it is unlikely to be helpful for the layman who wishes to gain a fuller understanding of the subject.

The latest issue of the *Philips Technical Review* (**23**, 1; October 1961) contains a description of a new digital computer *Pascal*. This machine, installed at the Philips Computing Centre at Eindhoven, has a well-balanced, parallel operation, arithmetic unit the speed of operation of which varies between 10 and 73 μsec. for the various operations. The machine is a binary one with optional floating-point facilities. As may be imagined from its origin, it has a large magnetic core store as well as drums, tape and a plugboard. Input and output are in punched tape or cards and on magnetic tape and the speeds considerably exceed those of standard telegraph and business machine practice. The components used in the machine include valves, transistors and diodes since, in addition to being a useful calculator, the machine will provide information on component performance.

Technical Papers of the Sandia Corporation

THE bibliography *SCR*-255 is a revision of *SCR*-150 and consists of a cumulative numerical list of technical papers of the Sandia Corporation, Albuquerque, which are available to the public (Pp. 120. Albuquerque, New Mexico: Sandia Corporation. Obtainable from the Office of Technical Services, Department of Commerce, Washington 25, D.C., 1961. 2.50 dollars). The prices of printed or photographic and microfilm copies are included in most cases. The title list includes in addition a section on engineering drawing and specifications on sale through the United States Atomic Energy Commission's Engineering Materials Section at Oak Ridge. The contents of the bibliography is divided into eight sections, dealing with the reprint and monograph series; technical reports; development reports; technical memoranda, divided into annual series covering 1953–60 inclusive; manuscript releases; miscellaneous papers; and engineering materials. The Sandia Corporation is a prime contractor to the Atomic Energy Commission.

Plasma Physics

REPORT No. 18 of the Danish Atomic Energy Commission Research Establishment, Risö, contains the essential part of the material presented in the form of lectures or at the seminars during the international summer school on plasma physics held there during August 1–12, 1960 (Pp. viii + 645. Risö, Roskilde: Danish Atomic Energy Commission Research Establishment, 1961. 60 D. Kr.). The lecturers were distinguished scientists from Denmark, France, Germany, The Netherlands, United States of America and Great Britain. The director was Dr. M. N. Rosenbluth (General Atomic, San Diego, California) and 130 students from twenty-four countries attended. The contents of the report, compiled by C. F. Wandel (Risö Establishment), consist of the short general introduction to plasma physics given by the director, followed by thirty-six articles grouped under the headings: single-particle motion, magnetohydrodynamics, fundamental equations, stability, relaxation, radiation, thermonuclear aspects, pinch and shock apparatus, concepts covering the stellarator and the mirror machine, and plasma waves and diagnostics. Not all the articles are in the form of lectures; some are reprints of published papers. The standard of the lectures, though representing a good introduction to and clear statement of the basic principles of plasma physics, nevertheless is somewhat more advanced than, for example, Lyman Spitzer's book. The report should prove an admirable text for postgraduate students and research workers who want to pursue an advanced course in plasma physics before proceeding to tackle the latest publications on the subject.

British Chemicals and their Manufacturers

THE Association of British Chemical Manufacturers has recently issued a guide, *British Chemicals and their Manufacturers*, which supplies information on all chemical products made by its members (Pp. 219. London: Association of British Chemical Manufacturers, 1961). The booklet is far more than a guide, and will be invaluable as a reference book to purchasers and producers alike. It will certainly prove to be indispensable in all academic and industrial research departments throughout the United Kingdom. The booklet consists essentially of five sections including the grouping of chemical industries, a directory of members of the Association, classified lists of British chemical products (with a special section on indicators and microscopical stains), lists of proprietary and trade names and proprietary and trade marks. The information is in each case presented in the clearest possible way. The first section provides information on the nature of the products, that is, their grade, whether heavy or fine, their origin, for example coal-tar distillation products, and their type such as dyestuffs, explosives, fats, glycerine, greases, soaps, etc., and those suitable for pest control. The directory, besides giving the name and address of members, also supplies telegraphic addresses, telephone and telex numbers. All in all, 366 industries are listed, and supplemented by a list of affiliated associations. Products are listed under their chemical names, and coded references give means of tracing the producer. In each case it is indicated whether the chemicals are of commercial grade or fine, pharmaceutical, pure, analytical and similar grades. The lists of proprietary and trade names will certainly be of great assistance to all editors who have to decide what is, and what is not, a registered trade name, and hence what should have quotation marks.

Autonomic Nervous System and the Occurrence of Visceral Lesions

Hiroshi Itoh, Teizo Kasahara, Kazua Amano, Koh Okamoto and Samon Tanabe, of the Department of Pharmacology, Yokohama University School of Medicine, have shown histologically that a chronic chemical stimulation on a defined region of the skin produced lesions in both renal cortices (*Yokohama Med. Bull.*, **12**, No. 3; June 1961). They have now followed the development of these lesions by functional examinations over a period of four months. The state of intrarenal circulation was investigated to determine the pathogenesis of the lesions, and the renal function was examined in several ways. The test for albumuria and Volhard's test proved to be positive, but disorders of renal function could not be detected by other methods. Albuminuria was con-

firmed at two stages of the experiment, namely, on the twenty-eighth and fifty-fifth days. Investigations made on the fifty-fifth and seventy-sixth days on the renal cortical region suggested some disorder of the intrarenal circulation. Over a period of four months the systolic blood pressure did not rise significantly above the level in the pretreated period. The authors suggest that the lesions may have been caused by impulses generated in the afferent nerve, following repeated chemical stimulation on the skin, passing through a reflex arc involving the higher central nervous system. These continue to the intrarenal arteries by way of the sympathetic nerve, and the resulting ischæmia in the renal cortex may be the cause of the degeneration of the renal parenchymal cells, especially those of proximal convoluted tubules. The functional changes of the kidney are slight in the period before the lesions advance but they are readily perceived by histological examination.

An Inventory of Abstracting Services

The Fédération Internationale de Documentation, 7 Hofweg, The Hague, Netherlands, is preparing, with financial assistance from the National Science Foundation in Washington, D.C., a comprehensive inventory of abstracting services covering the fields of science, technology and social sciences. It is expected that the study will take two years. The data will be collected by means of a questionnaire to be distributed at the beginning of February 1962. Besides gaining information about the nature and scope of each abstracting service, the questionnaire will also determine the number of periodicals consulted during the year, the existence of a reproduction service and whether translations can be obtained. On completion of this project, the Fédération plans to establish an international information centre, the first object being to give supplementary information on data revealed by the questionnaire. It is hoped that all abstracting services will co-operate in the scheme.

Wellcome Senior Research Fellowships in Clinical Science

To encourage medically qualified workers of exceptional ability to embark on careers of clinical research within the United Kingdom, the Wellcome Trustees are prepared to award one or two Wellcome Senior Research Fellowships in this field in 1962. The professors in charge of university departments of medicine, surgery and obstetrics in the United Kingdom are invited to put forward confidential nominations for these awards. Direct personal applications by candidates for these Fellowships will not be considered. The Fellowships will be awarded for one year in the first instance and will be renewable over four more years. It will be a condition of the appointment that the Fellow should be granted the status and responsibilities of a lecturer in the university department in which he is employed. On the assumption that the age of the Fellow at appointment will be about thirty, the stipend offered over the five-year period will be £1,900 a year, rising by increments of £150 to £2,500. Further information can be obtained from the Scientific Secretary, The Wellcome Trust, 52 Queen Anne Street, London, W.1.

Christmas Lectures for Young People

Among the lectures organized for young people during Christmas and the following weeks are: The one hundred and thirty-second course of Christmas lectures at the Royal Institution, to be delivered by Sir Lawrence Bragg on "Electricity", including "What is Electricity?" (December 28), "How Electricity Travels" (December 30), "Motors and Dynamos" (January 2), "Electrical Oscillations and Electromagnetic Waves" (January 4), "Our Electrical Supply" (January 6), and "Conveying Information by Electricity" (January 9). Further information can be obtained from the Royal Institution, 21 Albemarle Street, London, W.1. Five children's Christmas holiday lectures on "The Painter, the Anatomist and the Surgeon" (Mr. H. Thompson, January 1), "Science in Crime Detection" (Dr. K. Simpson, January 2), "Family Life in Russia To-day" (Dr. J. Hunt, January 3), "The Romance of Huntington's Chorea" (Dr. Macdonald Critchley, January 4) and "How and Why We Hear" (Mr. T. Cawthorne, January 5) at the Royal Society of Medicine at 2.30 p.m. Further information can be obtained from the Assistant Secretary, Royal Society of Medicine, 1 Wimpole Street, London, W.1. A young people's lecture, to be delivered by Prof. M. Stacey on "Nature's Plastics" at the Plastics Institute on January 5, at 2.30 p.m. Further information can be obtained from the Plastics Institute, 6 Mandeville Place, London, W.1.

Announcements

Prof. N. R. Dhar, recently head of the Chemistry Department and director of the Sheila Dhar Institute of Soil Science, University of Allahabad, has been elected as a corresponding member of the Paris Academy of Sciences in the Chemistry Section.

Mr. P. S. Briggs, of T. N. and F. H. Briggs (Tanners), Ltd., Leicester, has been appointed chairman of the Council of the British Leather Manufacturers' Research Association in succession to Mr. E. A. Hutchings, who has left the leather industry.

Dr. Olivier Heroux has been appointed scientist-in-residence at the U.S. Naval Radiological Defense Laboratory, San Francisco, California. This is the first appointment under a new programme at the Laboratory for visiting distinguished scientists from other scientific communities. Dr. Heroux is a member of the Canadian National Research Council and will be at the Laboratory during leave of absence of one year from his post in Canada.

The annual meeting of the American Mathematical Society will be held at the Sheraton-Gibson Hotel, Cincinnati, during January 22–25. Further information can be obtained from J. W. T. Youngs, American Mathematical Society, Bloomington, Indiana.

Erratum. Dr. G. B. Robertson writes: In "Crystal and Molecular Structure of Diphenyl" (*Nature*, **191**, 593; 1961) the value of the constant in the asymmetric-unit plane equation was reported incorrectly due to a computational error. The correct value of this constant is 0·00864, the equation of the plane is:

$$-0{\cdot}30584\,X + 0{\cdot}55575\,Y + 0{\cdot}77305\,Z' = 0{\cdot}00864$$

and the perpendicular separation between the ring planes is ~ 0·017 Å. (not ~ 0·2 Å.). This separation is not statistically significant and the two rings are, therefore, coplanar within the limits of the experimental error.

NEMATOLOGY AT THE IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

ON October 23, at Silwood Park, Sunninghill, the new Shell Parasitology Building was formally handed over to the Imperial College of Science and Technology. A plaque inside the door explains that "These laboratories were erected with the aid of a generous benefaction from Shell International Chemical Co., Ltd., in support of research into parasitology".

The new building consists of two floors, each of 2,400 sq. ft., and a small basement of 400 sq. ft. On the ground-floor are a lecture room and well-equipped teaching laboratory for the one-year postgraduate course in nematology, a preparation room for extracting nematodes from soils or from plant materials, and a radio-tracer room. On the first floor are three staff laboratories, a large laboratory for higher-degree students, a small teaching laboratory for undergraduates specializing in parasitology, and a photographic dark room. In the basement are three constant-temperature rooms separately controllable within the range 5°–30° C.

Fig. 1. Front view of the Shell Parasitology Building, Imperial College of Science and Technology

While research projects extend over the field of general parasitology (recent work has dealt with protozoal parasites of insects, and the bionomics of sheep trichostrongyle larvæ, for example), major prominence is given to nematology. The most pressing requirement was to house the postgraduate nematology course: it will now be possible to accommodate with comfort eight students, and at need up to twelve, instead of only four as hitherto.

This one-year course aims at giving a grounding in: (1) general nematology with an emphasis on plant-parasitic and soil-inhabiting species; (2) the principles of control by chemical and other means; (3) the art of experimentation. The course, the first of its kind in Great Britain, has a strong bias towards practical techniques and research methods. Of a total of fifteen students to date, ten have come from seven overseas countries, and it is hoped to maintain this overseas appeal, if only because plant eelworms are of great economic importance in tropical and subtropical lands.

The new laboratories are well equipped with such standard items as microscopes and calculating machines, and radiotracer equipment includes a versatile scaler with scintillation head, and monitor. Work is in progress with labelled chemical fumigants.

B. G. PETERS

THE HUMBER LABORATORY

THE Humber Laboratory of the Department of Scientific and Industrial Research at Hull held two open days during November 9–10. This establishment is a branch laboratory of the Torry Research Station, Aberdeen, and like its parent Station is concerned with research on the handling and processing of fish from the time of catching to retail sale. Since the Humber Laboratory was opened in 1952 it has dealt with an ever-increasing volume of work, particularly in applied research and in giving scientific information to the fish trade. The buildings have been extended twice during the short life of the station, and the recent open days marked the putting into service of a new block containing laboratories, library, and a lecture theatre.

The exhibits at the open days were not limited to the activities of the Humber Laboratory itself, but also covered the much wider range of subjects dealt with at the Torry Research Station. Prominent among these is the matter of freezing fish at sea. Poorer catches from present fishing grounds and the loss of some traditional grounds by the extension of territorial limits are encouraging longer voyages by British distant-water trawlers. This in turn means that the use of ice is no longer an adequate method of preserving all the catch in an edible condition. Freezing and cold storage is one obvious answer to the problem of preservation, but the successful application of this technique has required the co-operation of scientists and engineers trained in widely different disciplines. This was illustrated by exhibits which range from techniques of showing the damage to muscle cells during freezing, and of estimating the extent of denaturation of proteins in cold store, to a model of freezing plant designed to work on small ships in rough weather. The work of the Torry

Research Station has been particularly concentrated on the design of trawlers which will freeze part of the catch rather than on factory ships to freeze and process all of it at sea, and a model of one of the first vessels of this type to operate commercially was exhibited.

When frozen fish is used on any large scale the problem of thawing for further processing becomes a matter of very considerable importance. The thermal diffusivity of wet fish is very much less than that of frozen fish, and hence the thawing of a block of frozen fish in warm air is a self-retarding process. Also the temperature gradient permissible in such a process is limited by the need to minimize the spoilage of the fish already thawed. This has led to examination of dielectric heating as a means of thawing, and a practicable technique has been worked out. A pilot plant of 6-kW. output operating at 36 Mc./s. was on show. In this, frozen fish was carried on a conveyor belt between the plates of a condenser and 2-st. blocks were thawed in about 15 min. Equipment of this type developed by the Torry Research Station has recently been put into commercial operation.

Despite the attention given to frozen fish, work on wet fish has been by no means neglected. A number of exhibits were concerned with the effect of temperature on spoilage by reference to the chemical and bacteriological changes involved. The Humber Laboratory has been particularly concerned with the temperature of fish throughout the chain of distribution and has conducted temperature surveys at all stages from dock side to retail shop. This work has entailed the development of a number of special instruments, notable among which is a spear-type resistance thermometer in which the resistance element is contained within a hypodermic needle. For making temperature measurements by thermocouple on board moving vehicles, the conventional electronic self-balancing potentiometer is not a very suitable instrument. It is too large and its power consumption is too great. The Humber Laboratory has developed a small, battery-operated potentiometer in which balance is indicated by a 'magic eye', and this has been used successfully for many measurements on lorries. Another simple and effective instrument is the 'temperature indicator' which shows a sharp change in colour when it exceeds a given temperature. This device consists of a tube of 1 ml. capacity containing two aqueous solutions separated by a frozen non-aqueous layer.

The Torry Research Station has long been concerned with the smoking of fish, and when the Humber Laboratory was opened most of the applied work on this subject was transferred to Hull. The 'Torry kiln' has made considerable strides towards becoming the conventional piece of apparatus for smoking fish. Examples of the wide range of smoked products that can be made in a Torry kiln were on show together with sectioned models of both Torry and traditional kilns. The Humber Laboratory has devoted considerable effort to the question of the more efficient production of wood smoke, and has developed a continuous, automatically controlled smoke producer which uses a fluidized bed of sawdust. Tests have also been carried out to evaluate the performance of various commercial smoke producers, and a number of these machines was on show.

The work on smoking has also led to the development of various new instruments. A simple robust smoke density meter operating on the usual lamp and photocell principle was exhibited together with a modification of it, the 'smoke density integrator', which indicates directly smoke density multiplied by time—a quantity of great importance in smoke curing. Although more recent background work has shown that the invisible vapours in wood smoke are of more importance than the visible particles in giving smoked products their characteristic odour and flavour, smoke density meters are still of very considerable value. A technique for measuring the concentration of vapours was also exhibited.

Much of the work of the Humber Laboratory which has been of the most direct value to the fish industry has depended less on the discovery of new facts than on the practical application of well-established knowledge. For example, very considerable assistance has been given in setting up quality-control schemes which are based on taste panel techniques developed at the Torry Research Station in past years. Similarly, much-needed advice on the best methods of packing fish in ice for inland transport is merely the application to fish of the well-known laws governing the transient state conduction of heat. Work of this nature is extremely necessary in a station of the Department of Scientific and Industrial Research which serves the needs of an industry which as a whole is technically backward, and in which scientific staff are found only in the largest and most progressive companies.

D. L. Nicol

FOOD AND AGRICULTURE IN DEVELOPING COUNTRIES

On October 13 the Nutrition Society held a symposium, at the National Institute for Research in Dairying, Shinfield, Reading, under the chairmanship of the director, Prof. R. G. Baskett, on agricultural problems in developing countries. The latest Food and Agriculture Organization review of food and agriculture* shows that the developed areas still have an abundance, even a surplus of agricultural products, while malnutrition and hunger are the order of the day in many less-developed countries. The past decade has seen a very considerable expansion in the world output of food. For example, the average annual combined output of wheat, rice and maize (including the Food and Agriculture Organization estimates for the U.S.S.R. and mainland China) rose from about 470 million long tons in 1948-52, to about 720 million long tons in 1959. This remarkable technical achievement rests far more on increased efficiency of production than on increases in crop area. The overall food output per head has risen, but this covers dangerous decreases in Latin America and Oceania, and little change in Africa, as well as considerable increases in Europe (where population growth has been slower than elsewhere), North America and the Near East. In the Far East, where food production per head fell sharply during the War years, the pre-war standard, low as it was,

* Food and Agriculture Organization of the United Nations, Rome. *The State of Food and Agriculture*, 1961. Pp. viii+177. (Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1961.) 10s.; 2 dollars.

has been restored by increases in food production of about 3 per cent per annum. The very considerable increases in food production claimed in China since 1950 are perhaps no more than an intensification of this general trend.

In the past two or three years, however, food output has been more static, and in 1961 (including the disastrous harvests reported from China) total production was probably no greater than in 1960. International trade and co-operation are moving part of the Western surpluses into the needy areas, but there are not enough railway wagons, ships, elevators and sacks in the world to touch more than the fringes of the problem. For the hungry countries, continued increase in their own output, and particularly in yield per acre, is the only answer. Most of them have already done a good deal, as the Food and Agriculture Organization figures show, but the great disparity in yield per acre between the best and the worst indicates that far more could be done, particularly in India and Pakistan, which harbour one-quarter of the world's cattle and where the crop yields are among the lowest in the world.

The Nutrition Society meeting devoted itself largely to this question, with particular reference to tropical areas. Prof. L. Dudley Stamp spoke on the climatic limitations to development in the tropics. He distinguished first between the monotonous climate of the humid equatorial regions and the alternately wet and dry climate of the very diverse regions which he included in the tropics proper. In neither area does lack of heat limit plant growth, but in the latter variability of rainfall, in amount and in timing, is often pronounced. North and south of these regions lies the tropical arid zone, taken by the United Nations Educational, Scientific and Cultural Organization to lie beyond the 20-in. (500 mm.) isohyets.

At tropical temperatures soils weather deeply and organic matter is rapidly oxidized, while leaching removes plant nutrients beyond the reach of crops, though not of trees. Prof. Stamp felt that shifting cultivation, with hoes (and weeds) and without ploughs or tractors, might represent the best scientific answer to the land management problem in the humid tropics. (Clearly the many successful examples of plantation production of perennial crops must represent at least an exception to this generalization, though certainly in high rainfall areas the removal of vegetation, even for a very short period, in land clearing, is bound to create difficult or even dangerous hydrographic situations.) In the dry areas, the accumulation of salts and the formation of pans, together with erosion, can produce arid semi-deserts. In all areas of seasonal rainfall the heaviest agricultural tasks of the year fall on man and beast when they are weakened by the privations of the hungry season, and during the rains there are risks of flooding. Water control and irrigation, Prof. Stamp believes, are essential to development in all three regions. The indirect consequences of the tropical climate such as deterioration of stored food, and the heavy burden of insect and other pests and diseases of plants, animals and man, are also important.

To Prof. A. H. Bunting (University of Reading), who spoke next on the problems of improving arable output, the impression left, though perhaps unintentionally, by Prof. Stamp seemed unduly pessimistic. In many developing countries encouraging increases in food production have been reported, and impressive yields have been achieved in some. There are two parts to the problem of improvement: the intensification of production and the development of new agricultural land.

Apart from water, the tropical environment is by no means inherently unfavourable for crop production. The highest agricultural dry-matter yields so far recorded in the world are in sugar cane in Hawaii (around 30 tons dry matter/acre/year). The poor yields of peasant production must be compared, not with those of modern scientific farming, but with peasant yields in medieval Europe. Intensification is to be sought through improvements in protoplasm, production, and protection. There are many examples of improvement of yield, quality and disease resistance in tropical crops by selection and breeding. Better production often requires better land use, and the abandonment of shifting cultivation, but this can only come as part of a general process of improvement, usually on a regional scale. On a smaller scale, improved rotations, fertilizers, timely and sufficiently dense sowing, and the use of mechanization (particularly at times of peak labour input) have all been shown to pay dividends. Crop protection, including weed control, with the help of agricultural chemicals is well established for export crops in many tropical areas. Seed schemes, in which dressed seed of improved varieties is supplied at sowing time by an official organization, already exist in cotton and other crops: the general extension of such schemes would be a powerful aid to progress.

The development of new agricultural areas was illustrated by photographs from the Sudan rainlands, where many millions of acres of land suitable for arable production lie unused for lack of perennial water supplies.

Dr. J. C. Shaw (Food and Agriculture Organization), speaking on animal production problems, estimated that the annual deficit of animal protein in human diets (which is especially marked in the Far East) is equivalent to 1·8 million metric tons of non-fat milk solids. A Food and Agriculture Organization panel assessing livestock problems has given the highest priority to nutrition, followed by disease and management, with breeding last. In cattle, better nutrition means better management of better grazing plus the conservation of cereal and legume feeds and of forage. (Clearly this means that animal production, especially in drier areas, depends entirely on successful arable farming, but the converse is not necessarily true, as those who have advocated "ley farming in the tropics" seem to believe.) Much work is needed on the improvement of forage species, particularly legumes, on the intake of food and its nutritional value, and on the nutritional requirements of cattle in the tropics. Since much of the feed in the dry season comes from nutritionally poor materials, means of conserving or increasing their feed value must be sought. For small animals, farming waste products are important, and formula feeds will have to be devised. Breeding, in rigorous climates, should be based first on investigations of the productivity and improvement of the existing livestock, and should not necessarily be based on crossing programmes using improved stock from temperate countries. Generally, in developing countries, the shortage is not of animal agronomists but of scientific information: more modern veterinary schools are needed.

Dr. Shaw's assessment of the priorities in animal production was closely supported by Mr. T. J. Lansbury, recently of the University College of Ghana. He, too, gave the highest priority to nutrition, which appears far more important in limiting the

output of West African cattle (and also of goats, sheep and poultry) at present than their inherent characteristics. Drought leads to shortage of feed and of water, and may lead to losses of 25 per cent of live weight in a single season. The improvement of water supplies by conservation requires planned use of grazing, while the problem of dry-season feed supplements has scarcely been tackled, partly because there is little information on the composition of animal feeds in the savannah regions. The West African ox does not seem to possess the superior digestive powers with which it is credited, and indeed digestibility is reduced when animals are fed on a low plane of nutrition, particularly in respect of phosphorus and protein. Basic work on all these matters, which would no doubt include studies of urea and other non-protein nitrogen sources, is needed on the spot.

Introduced varieties of pigs and poultry can do well in West Africa. Pigmeat is not in demand, but great expansion of egg production is possible if the birds are properly fed and cared for and not regarded as mere scavengers. An industry based on imported chicks is developing, but serious nutritional problems (protein, vitamins B and others, calcium and other minerals) remain to be overcome. An industry for feeding-stuffs could be based on local materials which are at present exported for the formulation of feeds in Europe.

The last speaker, Mr. R. S. Rack, based his contribution on his remarkable work in the practical development of native fishing industries in Northern Rhodesia. His address was notable for its active concern with the human problems of agricultural development. He pointed out that the people of undeveloped countries have their own technical and social adaptations to their environment, and that the technological basis of their economy cannot be altered in isolation from its personal and social context. This difficulty shows itself in attitudes to organization and work discipline (whether self-imposed or not) and to the employment of time, and in the obstacles often presented by traditional habits and customs. The simple poverty of the native fisherman, and his social and family obligations, may prohibit the accumulation of capital; the size of his undertaking may in any event be too small to allow a single technical step (such as the purchase of an outboard motor) unless his whole scale of operation can be altered to take full advantage of it. Other problems include the supply of fish, the availability of technically competent and equipped fishermen, and the existence or possibility of markets and suitable transport. These factors were illustrated by an account of the successful measures taken since 1951 to improve the supply of fish (whether fresh, as a staple food, or dried as a relish in a cereal diet) to the Rhodesian Copper Belt, which involved an ice plant, a boat-building school, a marketing advisory organization and the encouragement of African traders, and is to lead on to a fisheries school which will teach navigation, the handling of powered boats and book-keeping in addition to the techniques of catching and preserving fish. Another example arose from the development of a fishing industry among the traditionally non-fishing Tongas when they were resettled because of the construction of the Kariba Dam, and a third from the improvement of a sardine-type fishery on Lake Tanganyika.

In the discussion following the papers, an interesting food chain involving water hyacinth, pigs and fish was described by Prof. Stamp from Hong Kong, and some disagreement was evident on the usefulness of imported stock in the tropics.

The Food and Agriculture Organization report, already mentioned, also deals with human and organizational problems, on a regional scale, in two special chapters. One, on land reform and institutional change, surveys the situation in the main regions of the world and then discusses particular problems such as tenancy, financial support, marketing, settlement and community development. The second is on the organization of extension education and research in Africa, Asia and Latin America. Taken as a whole, the report is, as usual, a valuable and informative survey.

The meeting, and the report, justify a degree of optimism not only about the technical possibilities but also about the chances that they will be in part realized during the next two or three generations. The social and historical imponderables are too great to make any further prediction useful: but it is impossible to avoid the conclusion that a general limitation of human numbers is ultimately desirable if a measure of dignity and worthiness of human life is ever to be achieved.

A. H. Bunting

SCANNING TECHNIQUES IN BIOLOGICAL AND MEDICAL MICROSCOPY

A CONFERENCE on the principles and applications of scanning methods in the microscopy of biological and medical specimens, arranged by the New York Academy of Sciences, was held in the Henry Hudson Hotel, New York, during October 5–6. Fourteen papers were presented in the four sessions of the meeting, and all but one were concerned with optical microscopy, the exception being on electron and X-ray scanning microscopy.

The main group of contributions ranged from the straight application of television in microscopical observation to the design of a complicated flying-spot system for sizing cells. M. J. Kopac (Graduate School of Arts and Sciences, New York University) described the use of closed-circuit television in connexion with the micro-surgical technique he has developed for operating on single cells. At present the micro-instruments, of which there may be as many as eight in use in a single operation, are manipulated by hand. It is planned to adapt the electronic techniques used for tracking targets in the air so as to control the movement of microneedles and micropipettes with servo-motors.

Scanning techniques are especially useful for cytochemical studies of transient processes in cells. C. N. Loeser and S. S. West (School of Medicine, Western Reserve University, Cleveland, Ohio) have been using a highly sensitive 'Intensifier Image Orthicon' television camera to record fluorescence emission spectra from individual Ehrlich ascites cells, stained

with acridine orange. The average uptake of dye per cell has been determined and correlated with the spectral measurements. For quantitative investigations in the ultra-violet, two approaches are being followed. G. Z. Williams (National Institutes of Health, Bethesda) and R. G. Neuhauser (Radio Corporation of America, Lancaster), described the use of a new 'Vidicon' television camera sensitive to the ultra-violet as an image storage detector and for time-lapse work. They obtain oscilloscope tracings of the changing ultra-violet absorption of single living cells, allowing deductions about reaction-rates. On the other hand, V. K. Zworykin and C. Berkeley (Rockefeller Institute, New York) have been exploring applications of the colour-translating television microscope. In this instrument, the specimen is illuminated by three selected ultra-violet wavelengths and the three images produced are electronically converted into images in three different colours on a television screen. It is being used for examining ultra-violet radiation damage, the streaming of protoplasm and the absorption of metabolites such as vitamin A.

A still wider range of application is open to the flying-spot technique, in which the specimen is explored point by point sequentially. The source of illumination is the flying spot on the screen of an oscilloscope, which is demagnified through the microscope to throw a moving probe on the specimen. The image is built up on a display tube, or the signal may be used directly for quantitative absorption measurements. With suitable phosphors any part of the spectrum can be used, or indeed soft X-rays. T. O. Caspersson (Karolinska Institutet, Stockholm) gave a masterly survey of the methods which can be used, and the factors influencing instrumental design, in the three main areas of investigation: analysis of single cells or cell structures with high precision, analysis at lower accuracy of large numbers of cells or cell structures, and investigations on living material, which are often only possible by semi-quantitative or even qualitative methods. He described a number of instruments in use, or in development, in his own laboratory for these different types of research, and discussed the choice between mechanical, optical and electronic systems of scanning.

Most of the remaining contributions were variations on these themes. J. G. Hoffman (University of Buffalo) described measurements of the nucleic acid content and the dimensions of mammalian cells and their nuclei, both by ultra-violet absorption in fresh cells and by absorption in the visible range in unfixed cells and in Feulgen-stained cells. He discussed the use of a digital computer to facilitate analysis of the absorption measurements as they are obtained. Britton Chance (Johnson Foundation for Medical Physics, Philadelphia) described investigations of respiratory enzymes by analysis of the fluorescence spectra excited in single cells. J. J. Freed and J. L. Engle (Institute for Cancer Research, Fox Chase, Philadelphia) discussed the advantage of using a vibrating mirror to produce the scan, since it allows monochromatic illumination at any desired wave-length. They described the instrument they have built for making repeated absorption measurements on single living cells. It was found that continuous exposure of ascites tumour cells for 30 min. at wave-lengths above 2400 Å. produced no observable radiation damage, whereas at 2400 Å., or below, the typical radiation damage syndrome was observed.

The chairman of the conference, P. O'B. Montgomery, who has been mainly responsible for pioneering flying-spot microscopy in the United States, described recent work in his laboratory (University of Texas Southwestern Medical School, Dallas). In addition to the development of a flying-spot interference microscope, he and his colleagues (W. A. Bonner and L. L. Hundley) have worked out techniques for simultaneous illumination of the specimen with both visible and ultra-violet light. In one version of the apparatus, the two images appear side by side on the display tube. Alternatively, a very small part of the specimen is imaged by ultra-violet while a larger field around it is imaged by visible light. In this way the progress of ultra-violet radiation damage on selected cells, or even on different parts of a single cell, can be followed.

One of the important uses of the flying-spot microscope is for counting and sizing particles, and instruments are commercially available for routine measurements of this type. Attempts are now being made to extend the technique to the 'recognition' and counting of specific types of cells in tissue sections or smears, by applying to microscopy some of the experience gained in the use of radar for target spotting. An instrument named the 'cytoanalyser' has been built for the mass screening of cervical and vaginal smears, to aid in the early diagnosis of cancer. It is best described as an analogue-digital computer connected with an electromechanical scanning system, and is the fruit of collaboration between the Sloan-Kettering Institute for Cancer Research, New York, the American Cancer Society, the National Cancer Institute, and Airborne Instruments Laboratories of Deer Park, New York. Its general principles of construction were described by W. E. Tolles and H. P. Mansberg of the last-named, and its functions and preliminary results of its operation by Elaine Diacumakos and Emerson Day of the first-named institution. The biological situation is complex, and it seems to be too early to judge how successfully the instrument can replace the human eye in spotting aberrant cells, and so dispensing with the squads of observers now employed in checking smears under the microscope. In any event, these two papers were another striking illustration of the feeding back of experience from the field of war to the problems of peace.

The remaining contribution, by V. E. Cosslett (Cavendish Laboratory, Cambridge), described the extension of flying-spot methods to the X-ray end of the spectrum, with some reference to the related technique of scanning electron microscopy. Both have so far been mainly applied to metallurgical and mineralogical problems. Very recently A. D. G. Stewart (Engineering Laboratory, Cambridge) has built an ion gun into an electron scanning microscope, to produce etching of a specimen surface during continuous electron imaging. It seems likely to be useful in studying the structure of teeth and other mineralized tissues, as well as of metals and alloys. At the Cavendish Laboratory, an exploration is being made (with aid from the Medical Research Council) of the possible biological and medical applications of the X-ray scanning microscope—more accurately described as a microanalyser. Some preliminary results were reported to the Conference, from the detection of foreign particles in lungs and other tissues to work on the mineralization of bone and teeth. An appreciable proportion of iron (up to 7 per cent) has been found in the surface enamel

of the incisors of a number of rodents. The technique has obvious uses in identifying dust particles in atmospheric pollution and related problems. The recent extension of its scope to the lighter elements, beryllium and carbon, makes an investigation of the possibilities of organic analysis desirable. However, it is doubtful if it can be of value in cytochemistry until the spatial resolving power is improved appreciably beyond the present limit of 0·25μ and the detection limit to better than one part in ten thousand.

The conference was throughout stimulating, and provoked much lively discussion, which often led back to fundamentals. It was the first meeting of its kind, and very much justified the initiative of the organizers. V. E. Cosslett

TIME-RESOLVED SPECTROSCOPY

A CONFERENCE on "Spectroscopic Studies using Time-Resolved Techniques" was held in Manchester on June 16, under the auspices of the Institute of Physics and the Physical Society.

A brief introductory address was given by Prof. H. S. Lipson, president of the Manchester Literary and Philosophical Society, who welcomed members of the conference to the new rooms of the Society. He pointed out that the meeting was the first to be held there by the Institute of Physics and the Physical Society since these new buildings were completed, and which indeed had only been opened in September 1960.

The joint secretaries of the conference were Dr. L. Bovey, Atomic Energy Research Establishment, Harwell, and Dr. A. H. Gabriel, Atomic Weapons Research Establishment, Aldermaston. On the programme seven communications were listed and an eighth was added by Dr. C. Breton, who described some recent French work on plasma. As in other recent international conferences the scope of time-resolved spectroscopic work on plasmas, or indeed on discharges in general, has been shown to have extended very widely since the end of the Second World War, at which time very little work in this field had been done. This new work has, of course, largely been prompted by the rapidly growing interest in high-temperature plasmas, partly because of the widespread interest in the possibility of obtaining thermonuclear reactions and, more recently, partly because of work on the so-called magnetohydrodynamic conversion of energy. In both these closely related fields spectroscopic techniques are pre-eminent. This is because such techniques do not normally interfere with the discharge being investigated and because of their great range and power. The need for time-resolved studies, often on a sub-microsecond scale, has also led to the development of advanced optical techniques, and the latter were very much in evidence at the present conference.

Although speakers from France, the United States and South Africa all made contributions, the majority were provided by various workers from the Atomic Energy Authority at Harwell and Aldermaston.

The first paper was given by Dr. A. H. Gabriel, who has been responsible at the Atomic Weapons Research Establishment for the development of much striking optical apparatus for plasma work. He spoke about a system incorporating a fast-rotating mirror camera and a medium quartz spectrograph, used, for example, in work on fast pinched discharges with electron densities varying in the region 10^{15}–10^{18} per c.c. and lasting a few μsec. and time resolutions of the order of 0·1 μsec. or less. Dr. Gabriel then proceeded to describe various other optical techniques available, namely, an image-rotating device in which three mirrors are used with a vertical slit, and an apparatus for work in the vacuum ultra-violet region using near normal incidence and covering the wave-length range 2000–5000 Å. In such an instrument it was pointed out that astigmatism of the concave grating presents a difficulty. The spectrum is recorded near the base of the normal on to the Rowland circle and since only a limited wave-length range can be covered on one setting the position of the entrance slit assembly is made movable. A resolution of 3-mm. image displacement per μsec. is obtained. In connexion with all this work a precision capillary light source for calibration in the visible and quartz ultra-violet has been developed which gives virtually uniform illumination for about 12 μsec. and with an effective black-body temperature of about 25,000°. Another source for vacuum ultra-violet work, based on that of W. R. S. Garton at the Imperial College of Science and Technology, was also described by Dr. Gabriel. Experiments at the Atomic Weapons Research Establishment that were mentioned include measurements of excitation temperatures, that is, state populations, etc., for various discharges, and studies of free-free continua in the temperature-range 10^5–2×10^6 degrees, and finally, Dr. Gabriel mentioned experiments involving observation of emission spectra of rapidly pinched discharges, for example, the influence of impurity lines at different times, and work on absorption spectroscopy using axial viewing from a hollow pinch in which electron densities could be obtained from a study of line widths.

The next speaker, Prof. J. D. Craggs of the University of Liverpool, described recent experiments on the measurement of line broadening due to the inhomogeneous field Stark effect in spark discharges (transient arcs). He briefly summarized the historical development of this technique, which is now widely used in plasma investigations, from the days when the statistical theory was considered adequate to the present time when a more accurate realization of the complexities of the effect, particularly with regard to the contribution made by electrons in the impact broadening process, has been reached. Recent experiments in Liverpool, notably by Dr. E. E. L. Mitchell, strongly support the recent theoretical work of Griem and Kolb in the United States. This is for the Balmer lines of hydrogen, but in view of the present importance of the impact broadening contribution of electrons, experiments have been carried out over the past two years in Liverpool by Mr. Lim, who has simultaneously studied sodium and argon lines (notably the former) broadened by the impact effect and the statistical-impact broadening of the Balmer lines. These results again support the Griem–Kolb theory and direct attention to the possibility of making electron density measurements

with the use of impact-broadened lines. The use of sodium–hydrogen discharges has led to a number of interesting excitation effects which are being studied and Prof. Craggs briefly referred also to measurements of de-ionization of plasmas, with electron densities varying from about 10^{16} to 10^{18} per c.c., in relation to the recent work of Prof. D. R. Bates and Dr. A. E. Kingston in Belfast and Dr. R. W. P. McWhirter in Harwell.

Dr. F. D. Harrington of the United States Naval Research Laboratory proceeded to describe a series of time-resolved spectrographs developed and used in recent years at that Laboratory. In particular, the applications included investigations of exploding wires and high-velocity luminescent plasmas. The instruments broadly fall into three general groups: (*a*) the moving film type; (*b*) the rotating drum type; (*c*) the rotating mirror type. Instruments in the first category are somewhat unusual in this respect, at least in European studies, and the technical excellence of the design and optical work was very apparent. For example, Dr. Harrington described an *f*/6·6 ciné high-speed prismatic spectrograph for use in the near ultra-violet and visible region with film speeds of 9 ft./sec. and 90 ft./sec. giving respectively 460 and 4,600 frames/sec. The resolving power was about 5 Å. at a wave-length of 4000 Å. with a dispersion of 125 Å./mm. Another striking instrument described was a rotating drum continuous writing *f*/2·0 ultra-violet spectrograph. Experiments were then described on exploding wire spectra for a time-range 0–10 μsec. and using a 300-joule condenser discharge.

Dr. R. Wilson of Harwell spoke on time-resolved intensity investigation of spectral lines emitted by impurity ions in *Zeta* hydrogen plasmas and concentrated on recent work on interpretation of these spectra rather than on the instruments involved. The impurity lines were examined with monochromators covering the wave-length range 100–6000 Å. and most of the work fell in the vacuum ultra-violet region where the strong lines of highly ionized ions occur. Dr. Wilson stressed that in a plasma of this type in which the electron density is of the order of 10^{14} per c.c. at an equivalent temperature of 20 eV., the dominant processes are ionization by electron collision and radiative recombination, as in the solar corona, and this meant a strong departure from thermal equilibrium. Dr. Wilson described recent experiments on the transient lifetime for spectral lines corresponding to successive studies of ionization and showed how these varied in a partially contained plasma. Data were presented for the intensity transients of lines due to Ar I–Ar VIII. The time duration of the optical transients for these different states increased up to Ar VI but thereafter remained constant, and similar results were described for O IV, O V and O VI. Many new spectral lines, for example, due to Ne VII and Ne VIII, Kr V, Kr VI, Kr VII and Kr VIII and Xe V to Xe IX have been identified. The containment times for many of these highly ionized states have been studied. Thus, for example, in certain experiments Ar VIII was contained for about 450 μsec.

Dr. R. V. Williams of the Associated Electrical Industries Research Laboratories, Aldermaston, also described time-resolved spectroscopic work on toroidal plasma discharges such as those in the apparatus *Sceptre* 4. Dr. Williams described the range of techniques available at the A.E.I. Laboratories, including multi-channel recording with photomultipliers. Experiments have recently been done on *Sceptre* 4 on radial variations of electron temperatures, for example, by studying the 2822 Å. line of B IV which is concentrated near the centre of the discharge, and the 2066 Å. line of B III was observed farther out, that is, nearer the tube wall. These lines were excited when methane was added to the discharge in very small amounts, which are shown to have negligible effect on the overall properties of the discharge. Further radial work on the 2271 Å. line of C V (C V is helium-like) were mentioned. Various investigations of this kind indicated, for example, electron temperatures kT_e of the order of 20 eV. Dr. Williams also mentioned in this connexion new work in progress on F V and F VI lines which is being carried out again to obtain electron temperatures. Plasma instabilities are being detected spectroscopically by examining high-frequency intensity fluctuations in spectral lines; the frequencies involved lie in the range 10–100 kc./sec. and instabilities occurring in different parts of the discharge could be separately detected by observing lines from ions having varying ionization potentials. For example, the 2781 Å. line of O V was used for examining instabilities near the centre of the discharge and the second Balmer line (D_β) for instabilities occurring near the wall of the containing torus. Further experiments involving the use of two monochromators so placed that each gave one of a pair of oscillograms of a single line, have given some support to recent theoretical treatments of plasma instabilities which indicate that the latter should be propagated in a torus with velocities depending on the magnetic field and the plasma pressure, the measured velocities of such instabilities agreeing quite well. Predicted values were about 10^6 cm./sec.

Dr. N. J. Peacock of the Atomic Weapons Research Establishment Plasma Group at Aldermaston described spectroscopic work on the fast pinch discharges at that establishment. The experiments involve rapidly rising axial magnetic fields, electron densities of the order 10^{16} or 10^{17} per c.c. and electron temperatures estimated, for example, from measurements of the continuum established in the wave-length region below 20 Å. as 100 eV. or higher. Time-resolved spectrograms provide evidence in support of other work with streak cameras and with X-ray and neutron emission studies when maximum temperatures are reached during the second half-cycle of the driving field. Time-resolved spectrograms have enabled temporal variations of electron density, ion and electron temperatures and plasma distribution to be made on helium discharges and other discharges including a few per cent of added impurities. Studies of Stark-broadened profiles of the He II line 4686 Å. agree well with Griem's theoretical work. Time-resolved spectroscopic work on electron temperature and electron density taken for various radial positions in the tube enable diffusion-rates of the plasma through the containing field to be estimated. Dr. Peacock also described emission work on deuterium–oxygen discharges, for example, in which energy balance relations have been studied. In this work electron temperatures have been estimated from the ratios of intensities of He I and He II lines and in various other ways. Dr. Peacock showed interesting space-resolved spectra for helium and temporal variations of ion density across the discharge showing a shell structure.

Mr. W. W. Schroeder of the National Physical Research Laboratory, Council for Scientific and Industrial Research, Pretoria, described recent developments

in photo-electric recording of spectra from transient sparks. The techniques have been developed specifically to improve time-resolved spectroscopic analysis of solids, for example, the quantitative detection of copper in aluminium alloys. The controlled gating of the photo-multiplier proved to be so good that special controlled spark sources had to be developed and a description of such a source was given based on the work of A. Bardocz in Hungary. This source used an air-blasted control gap in series with the test gap. The jitter proved to be not more than 15 mμsec. over 10^7 discharges, and the importance of ultra-violet illumination of the test gap by the control gap was shown to be important.

It was possible to analyse a spectral line with a resolving time of 0·1 μsec. The speaker stressed that in spectroscopic analysis of this kind time-resolved observations might well be of importance in the investigation of analysis and internal standard lines, etc.

Dr. C. Breton (Fontenay-aux-Roses) gave an account of spectroscopic observations made in that establishment, including the observation of impurity lines in the far ultra-violet in a toroidal discharge and a new technique for the study of line profiles. The former work was carried out with a vacuum grating spectrograph using seven exit slits, each with a separate photomultiplier tube. Impurity lines from carbon, oxygen and nitrogen ions were observed with a 20 kV. 200 microfarad capacity bank feeding toroidal discharges at a pressure of 10^{-3} mm. of mercury. Dr. Breton described a new and interesting technique in which a broadened line profile is divided into thin slices by the use of successive layers of glass fibres some 50μ in diameter to centres of spaces approximately 55μ apart. The assembly is used in a 1-m. focal distance monochromator giving an average dispersion of about 3 Å./mm. in the first order and in the visible region. Each fibre corresponds to an interval of about 0·15 Å. and Dr. Breton showed curves giving temporal variations of the profile of H_β; finally, he mentioned experiments on plasma radiation and the drift velocity of ions in a magnetic mirror machine.

J. D. Craggs

ELECTRON SPIN RESONANCE

On November 2 the Chemical Society held a symposium at Queen Mary College, London, on electron spin resonance. In the first part of the symposium there were three papers. In an introductory paper, Prof. H. C. Longuet-Higgins outlined the principles of electron spin resonance measurements. Only paramagnetic substances show this phenomenon, which arises from their unpaired electrons. In a magnetic field the two spin states of a single electron are split apart in energy by virtue of the magnetic moment of the electron. An oscillating magnetic field, applied at right angles to the permanent field, causes the electron to jump from one of these levels to the other if the frequency ν of the oscillating field satisfies the relationship:

$$h\nu = g\,\beta H,\ \beta = eh/4\pi mc$$

Here H is the strength of the permanent field and g is a number which equals 2·0023 for a free electron. Electron spin resonance measurements acquire their interest from two subsidiary physical effects. The first of these is the so-called hyperfine interaction, which arises when the paramagnetic molecule includes magnetic nuclei. The value of g then depends on the orientation of all the magnetic nuclei, and a series of lines is observed rather than a single resonance. As an example, n equivalent protons will give rise to $n + 1$ hyperfine lines with a binomial distribution of intensity; a single nucleus of spin I (which may be $\frac{1}{2}$, 1, $\frac{3}{2}$, etc.) will give a set of $2I + 1$ equally spaced lines of equal intensity. The foregoing remarks apply to a paramagnetic molecule tumbling over and over in the liquid phase. But if the molecule is fixed in the external magnetic field, the spectrum may be more complicated because of direction-dependent interactions between the electron and the magnetic nuclei. The directional variation of the spectrum may then enable one to identify the orbital in which the odd electron is moving.

The other physical effect which provides useful information about the nature of the unpaired electrons is spin-orbit coupling. An electron with orbital angular momentum is easier to orientate in some directions than in others, and this means that g must be regarded not as a pure number but as a tensor. The axes of the g tensor must coincide with the symmetry axes of the molecule, and certain applications of this fact would be discussed later in the symposium.

The next paper, by Prof. S. I. Weissman, was concerned with line-widths in electron resonance spectra. The width of a line is inversely proportional to the mean life-time of the spin states involved in the transition. Measurements of line-broadening can therefore throw light on the rate at which electrons are transferred from one molecule to another (assuming this to be the process which determines the life-time). Prof. Weissman gave some examples, including the reaction of the naphthalene molecule with its negative molecule ion and the exchange of a sodium ion between two naphthalene molecule ions. As he pointed out, an electron resonance spectrum always supplies kinetic information, whether one is interested in it or not.

The third paper, by Prof. D. J. E. Ingram, was concerned with the structural information which is provided by electron resonance measurements on single crystals. Prof. Ingram classified this information under three headings: measurements on the g tensor, studies of the hyperfine structure and measurements of electronic splittings (sometimes called zero-field splittings). At Southampton he and his colleagues had determined the orientation of the hæm in groups in myoglobin and hæmoglobin by examining the way in which g varies when the crystal is rotated in the externally applied magnetic field. In this particular case there is a very large variation in g, from 2 to 6, so that the orientation of the hæms can be very accurately determined. In myoglobin there are two hæms per unit cell and their orientation as determined in this way was afterwards confirmed by the X-ray work of J. C. Kendrew. An interesting molecule is hæmoglobin azide, where the g

tensor is completely anisotropic. J. S. Griffith had been able to determine from this tensor the order and spacing of the 3*d* orbitals on the iron atom; this order indicates very strongly that the histidine group attached to the iron atom is in a plane parallel to one of the diagonals of the hæm plane.

Information can also be obtained from the hyperfine splittings produced by protons or other magnetic nuclei. These splittings can be correlated with the types of group that are present and the angles which they make with one another. In order to extract such information from the data, one must, however, make use of molecular orbital theory, and the same applies to the sort of information which can be obtained from measurements of zero-field splittings.

In the second section of the symposium, four papers were presented.

The first paper, by J. R. Bolton and A. Carrington, was concerned with two topics. The first was the measurement and interpretation of the electron spin resonance spectra of the negative ions of toluene, *p*-xylene and *m*-xylene. These are of special interest because of the degeneracy of the lowest antibonding orbitals of benzene. This degeneracy is removed by introducing methyl groups, and one can predict from the molecular orbital theory which of the two orbitals is likely to be occupied in the ground-state of each ion. The spectra of these xylenes, and of their partially deuterated isotopes, completely confirm the theoretical predictions, and in particular the hypothesis that the methyl groups repel the additional electron.

The other topic discussed by Dr. Carrington was certain remarkable line-width alternation effects which occur in the electron spin resonance spectra of the durosemiquinone positive ion and in the positive ion of 5,8-dihydroxy-1,4-naphthasemiquinone. This effect is almost certainly due to *cis-trans* isomerization proceeding at a rate comparable with the separation (in cycles/sec.) between the hyperfine lines in the resonance spectra. Dr. Carrington also directed attention to the possibility of determining the signs of hyperfine interaction constants by studying the variation of line width as one proceeds from one end of a spectrum to the other.

In the next paper Dr. E. A. C. Lucken described experiments on various substituted *p*-benzosemiquinones and polynuclear aromatic quinones. He used these measurements in attempting to evaluate coulomb integrals and resonance integrals for the various types of atoms and bonds. These attempts were not altogether successful, but Dr. Lucken claimed that his experiments could be interpreted by supposing that the methyl group produced an inductive effect, but did not enter into hyperconjugation. In the subsequent discussion, Dr. Carrington said that he, J. R. Bolton and A. D. McLachlan had obtained strong evidence for hyperconjugation—at least in hydrocarbon cations—from experiments on the positive and negative ions of anthracene and its 9-methyl and 9,10-dimethyl derivatives.

Prof. M. C. R. Symons then gave a lecture on the use of electron resonance in identifying the products of radiation damage in some inorganic oxy-salts. As Prof. Symons pointed out, such work can provide interesting information about the closed-shell species from which the paramagnetic radicals or radical ions are derived. He illustrated this remark with reference to the manganate and hypomanganate ions which result from the addition of one or two electrons to MnO_4^-. Work on damaged crystals is, however, often complicated by the difficulty of identifying the products, especially if the common isotopes of the nuclei have no magnetic moment. Nevertheless, in some cases it may be possible to argue by analogy, as in the series ClO_3, SO_3^-, PO_3^{--}. Prof. Symons described some preliminary work on the oxyions of chlorine, nitrogen and sulphur, and offered an explanation for some apparent discrepancies between the results of various authors who had studied the NO_2 molecule in different environments.

The final paper in the symposium was given by J. R. Morton. When a crystal of L-alanine is irradiated with γ-rays the radical CH_3CHCO_2H is produced. The electron spin resonance spectrum of this radical shows hyperfine structure which varies with the orientation of the crystal and also changes markedly when the crystal is cooled to 77° K. It is possible to analyse this spectrum fully and to obtain the hyperfine coupling tensors for the CH proton and the three protons of the CH_3 group. The radical is found to be planar, with a CH_3—C—H angle of 121 ± 3°. The spectra at 77° K. indicate that the methyl group has ceased to rotate at that temperature, and an estimate of the height of the rotation barrier can be obtained from the spectra at intermediate temperatures.

At the end of the meeting Dr. D. H. Whiffen proposed a vote of thanks to Prof. K. W. Sykes for arranging the symposium and to Queen Mary College, London, for generous hospitality.

H. C. Longuet-Higgins

THE HEALTH OF SEAFARERS

THE total number of seafarers on ships other than those engaged in coastal trading is in the region of 750,000. They form a definite community the members of which are linked together by mutual interest—and health hazards—at least as closely linked as they are to their parent countries. Although much has already been done to improve their working conditions and health standards, much remains to be done. In 1959 the World Health Organization undertook a study of the whole problem of the health of seafarers. As a preliminary fact-finding step, the World Health Organization Regional Office for Europe convened a conference at which it became clear that not enough was known of the health problems of seafarers or of the health services available to them. Following this conference, a questionnaire was drawn up by the Organization in consultation with the International Labour Organization and circulated to member States. The next step was to appoint a consultant to visit some of the major ports to analyse the problem in those ports where services were already in being and to supplement, where necessary, the information given in replies to the questionnaire. The ports visited were Liverpool, London, Marseilles, Athens, Alexandria, Bombay, Singapore, Manila, Hong Kong, Tokyo, San Fran-

cisco, Montreal, Helsinki, Gothenburg and Hamburg. The findings of this investigation were reported to a Joint Committee on the Hygiene of Seafarers, set up by the two Organizations, that met in Geneva during May 1–4, and are reported in a recent issue of the *WHO Chronicle* (15, No. 10; October 1961).

There seems to be general agreement that certain complaints occur more often than others, such as diseases of the gastro-intestinal tract, cardiovascular diseases, skin diseases, mental disorders, venereal diseases, tuberculosis and dental complaints. The accident-rate is high. Owing to the lack of comparative morbidity statistics it is difficult to ascertain whether accidents and these diseases occur more frequently among seamen than among male groups with the same age distribution in the general population. There is also some national bias in disease incidence. Venereal disease and tuberculosis remain common causes of incapacity in some of the developing countries, whereas mental disorders are more common in some of the developed countries. There is little information about the number of deaths occurring among seafarers, but accidents seem to be the commonest cause, accounting for more than 50 per cent of all deaths in the merchant service in some countries.

In certain countries there are no special hospital or clinic facilities at all for seafarers ashore. In these countries seafarers are referred to the general hospital or private medical care facilities available to the public. In the United Kingdom, Norway and Sweden, there are a few special clinics and hospitals based on some major ports and organized by the shipping companies and local authorities acting separately or in conjunction. Finally, there are a few countries which provide free medical care for seafarers as a special class; in the United States and Canada through the public health service, in Israel by a private company, in Japan by the Seamen's Insurance Association. In all cases, continuity of treatment from port to port or port to ship is poor.

Some Governments have no definite regulations regarding the provision of a ship's doctor. Most stipulate a physician if there are more than 100 persons aboard, and many a nurse or nurses also if passengers and crew exceed a given number. A few countries require a doctor for less than 100 persons. When, for any reason, no physician is available, medical care is provided by the master or chief officer, occasionally by another designated officer or by a nurse; in many countries the responsible individual is given special training for the work, though such training is not always of a high standard. The ships of most countries must carry a medical guide and a medical chest, but the standards of equipment and inspection are sometimes inadequate. Some States have no form of health insurance, either for the general public or the seafarer; in others, health insurance is included in a comprehensive scheme. In some cases there are special compulsory arrangements for seafarers.

Despite the defects reported and the apparent chaos of national regulations and facilities, much has been done to ensure certain minimum standards. A great deal of useful work is done by the Red Cross and other voluntary bodies and by the shipping industry itself.

Several conventions and recommendations concerning the health of seafarers have been established by the International Labour Organization. These deal with medical examinations, medicine chests, medical advice by radio at sea, social security and sickness insurance, and can be extremely helpful to countries developing their health services for seafarers. Not all these conventions and recommendations have been universally adopted, or fully implemented where they have been adopted.

A good deal can be done towards the prevention of injuries and disease on board ship. Attention to design and to lighting at strategic points will help to limit accidents; instruction in the composition and preparation of food is important to prevent gastro-intestinal disease; and periodic medical reviews have their place in the prophylaxis of mental illness, dental disease, venereal disease and tuberculosis. The development of a system to produce reliable statistical data to fill existing gaps in the morbidity and mortality statistics on seafarers would be helpful in deciding what type of medical organization should be set up for them on an international basis.

There is also the question of hazards on nuclear-powered ships. Few of these vessels are in commission at present, but there will be many more. The point at issue is not the danger to the public from a major accident, but the specific risks to the crew. It is essential to have a skilled man acting as radioactivity monitor, and protective clothing should be available for all crews. Periodic examinations for signs of radiation sickness are as necessary as at nuclear plants ashore. All nuclear-powered ships should be clearly marked so as to be easily identifiable in case of collision or other accident.

EFFECT OF MECHANICAL STRESS ON SOME SOLID-STATE POLYMERIZATIONS

By Dr. C. H. BAMFORD, Dr. G. C. EASTMOND and Dr. J. C. WARD

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FOR some time workers in the field of free-radical reactions in the solid state have suspected that an important part of any mechanism will involve crystal imperfections and annealing processes[1–4]. In this case it might be expected that mechanical stresses would exert a noticeable effect on the course of the reaction. According to Adler's views[1,2], based on different considerations, a compressive stress would lead to an increase in the rate of reaction, since it would tend to reduce the strain-energy at the monomer–polymer interface[1], polymerization usually being associated with a decrease in volume.

We have been examining the polymerization of crystalline acrylic and methacrylic acids (m.p. 12·5° C. and 16° C. respectively), initiated by ultra-violet light, and hope to report on recent work

shortly. The purpose of this communication is to record some novel observations.

The specimens used were in the form of thin layers of non-overlapping crystals, approximately 10μ thick, formed by allowing degassed monomer to freeze in the narrow gap between a flat silica disk and a glass microscope coverslip. The monomer samples had a water content of less than 0·02 per cent. The crystals were formed and contained in a cell fitted with a quartz window and with provision for evacuation and cooling. By the compression of springs suitably located within the cell an even mechanical pressure of about 50 lb./sq. in. could be applied normal to the layer of crystals, at any stage of the reaction, without disturbing the sealing of the cell. The specimens were irradiated by ultra-violet light from a high-pressure mercury lamp, the cell being situated in a thermostat tank at 4° C.

The course of the reaction was followed by observing the crystal birefringence, which decreased during irradiation. A plot of birefringence against time was sigmoid in shape, showing marked autocatalysis for both monomers. No effects other than the decrease in birefringence were observed, at least in the early stages of reaction. The occurrence of polymerization was confirmed by raising the temperature above the melting point of the monomer, when polymeric pseudomorphs of the original crystals could be seen.

In the absence of pressure extensive polymerization of crystalline acrylic acid, indicated by a complete loss of birefringence and absence of liquid formation on warming, was achieved after 10 hr. irradiation. In otherwise identical experiments in which the compressive stress was applied throughout, only a trace of polymer was formed after 20 hr. irradiation; further, observation of the birefringence during this time showed a small decrease in the initial stage with no subsequent change throughout the time of irradiation (Fig. 1, curve *a*).

Fig. 1, curve *b*, shows the effect of application of stress during irradiation. The crystals of acrylic acid had been irradiated without stress for 5 hr.; application of compressive stress then caused complete cessation in the decay of birefringence. When this stress was afterwards removed the reaction started again, being preceded by an induction period similar to that observed initially.

Methacrylic acid crystals behaved in an analogous way, with differences in detail. When pressure was

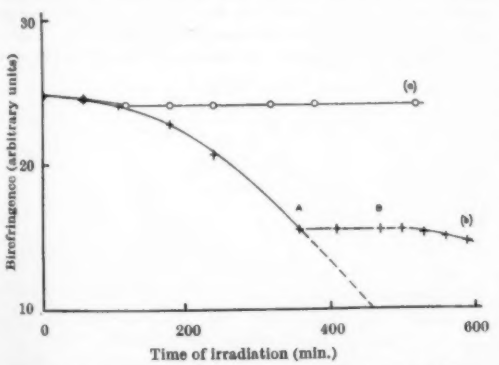

Fig. 1. Polymerization of acrylic acid crystals at 4° C. in ultra-violet radiation. (*a*) Stress applied throughout period of irradiation; (*b*) stress applied at *A*, released at *B*; – – –, normal course of reaction without stress

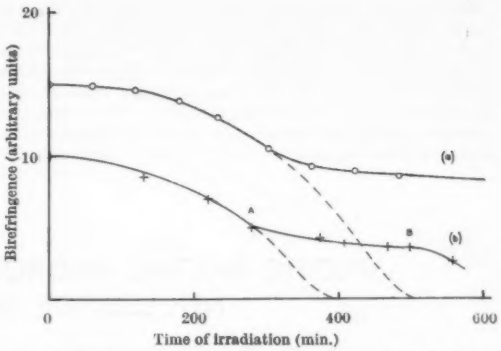

Fig. 2. Polymerization of methacrylic acid crystals at 4° C. in ultra-violet radiation. (*a*) Stress applied throughout period of irradiation; (*b*) stress applied at *A*, released at *B*; – – –, normal course of reaction without stress

applied at the beginning of irradiation the course of the reaction followed that without pressure for about 4 hr., but the rate of decay of birefringence then declined and complete loss of birefringence was only achieved after a period of irradiation exceeding 24 hr. In the absence of pressure, complete conversion is attained in a much shorter time (Fig. 2, curve *a*).

Application of stress to methacrylic acid crystals during irradiation, at a conversion of about 50 per cent, caused a marked and progressive reduction in the rate of loss of birefringence (Fig. 2, curve *b*), which eventually fell to an extremely small value. Removal of the stress produced an immediate acceleration. There was some indication that the effect of pressure was anisotropic, different crystal aspects being affected to different extents.

These results show that mechanical stress can have a large effect on the photopolymerization of crystals of these acids. The pressures used in this work are relatively very small—about two orders of magnitude less than those required to affect some solid-state phenomena[5]. In this connexion it is relevant to note that the reaction temperature was not far removed from the melting points; acrylic acid, which shows the greatest effect of pressure, was 8·5 deg. C. below the melting point, compared with 12 deg. C. for methacrylic acid. Further, it is apparently necessary for some polymer to be formed before the sensitivity to pressure is developed.

There appear to be three main types of explanation which can be advanced to account for these results. First, a macroscopic second phase—liquid or vapour—may be necessary for polymerization, for example, situated initially between the crystal and the boundary surface; this could be removed by the application of pressure, which might 'squeeze out' the second phase, or cause it to freeze. When the specimens were prepared the liquid was seen to freeze completely, the crystals growing in contact with both boundary surfaces, and no evidence for the existence of such a second phase was ever observed. Further, the effect of pressure on the freezing points of these acids is small, of the order of 1 deg. C. for 100 atm. Secondly, the pressure might remove imperfections, for example dislocations, which are the sites of initiation and propagation. This process might be considered as a sub-microscopic analogue of that described here and would reduce the mobility of the molecules below the limiting value required for polymerization. Either of the above explanations

would imply that polymerization occurs in regions where there are discontinuities in preference to the perfect lattice. Thirdly, one might suppose that applied stress produces (rather than destroys) imperfections[5], which trap propagating radicals and reduce the rate of polymerization. However, the polymer produced itself constitutes an imperfection in the crystal, and since the reaction is autocatalytic one might argue that, in fact, imperfections facilitate the reaction.

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[2] Adler, G., Ballantine, D., and Baysal, B., *J. Poly. Sci.*, **48**, 195 (1960).
[3] Bamford, C. H., Jenkins, A. D., and Ward, J. C., *J. Poly. Sci.*, **48**, 37 (1960).
[4] Bamford, C. H., and Ward, J. C., *Fifth Intern. Symp. Free Radicals*, Paper No. 6, Uppsala, Stockholm (1961).
[5] Choudhury, *Naturwiss.*, **48**, 594 (1961).

SPECIFIC PARTIAL HYDROLYSIS OF NUCLEIC ACIDS IN NUCLEOTIDE SEQUENCE STUDIES

By Dr. D. W. VERWOERD, Dr. H. KOHLHAGE and Dr. W. ZILLIG

Max Planck Institute for Biochemistry, Munich

THE specific partial hydrolysis of the nucleic acid chain is one of the main problems confronting the investigator working on the sequential analysis of the nucleotides in nucleic acids. A method for the stepwise degradation of the chain, proceeding from one end and combining enzymatic and chemical procedures, has been developed by Whitfeld[1] and was recently improved by Zamecnik[2] and Cohn[3]. Though it can be applied successfully to end-group analysis or to the analysis of short-chain fragments, the method has the same disadvantages as purely enzymatic procedures, namely, insufficient specificity and non-quantitative yields. These factors impose a limit of a few sequences.

Up to the present, only methods of enzymatic nature have been available for the specific partial hydrolysis of the nucleic acid chain, such as treatment with pancreatic ribonuclease (RNase) and with the ribonucleases specific for adenylic and guanylic acids isolated by Egami[4]. We therefore investigated the possibility of modifying nucleic acids by chemical means in order either to achieve a chemical hydrolysis or to modify the known enzymatic degradations. Chemical hydrolysis would be made possible if the β-elimination principle described by Linstead[5] could be applied. Modification of the mode of action of pancreatic RNase would result if either of the two main pyrimidine bases in ribonucleic acid (RNA) could be removed or changed in such a way that RNase would no longer attack the adjacent 3′-phosphodiester bonds.

By this approach one would expect to obtain optimally three or four different hydrolytic procedures yielding fragments of rather small length with overlapping sequences. Since nucleic acid chains, often consisting of thousands of nucleotides, contain virtually only four different bases, it would, however, still be impossible to reconstitute the full sequence of a long chain, unless additional methods are devised for obtaining larger fragments.

With this in mind we have investigated the reaction of hydrazine and hydroxylamine with RNA and its constituents.

Levine and Bass[6] first described the reaction of hydrazine with pyrimidine nucleotides. They demonstrated a splitting of the ring in uridylic acid with the formation of free pyrazolone and a urea residue attached to the ribose. Witzel[7] used this reaction to prepare nucleoside 5′-phosphates from dinucleoside monophosphates containing uridine 3′-phosphate by degrading the uracil, removing the urea residue by weak acid hydrolysis and cleaving the resulting β-carbonyl phosphoric ester by alkaline hydrolysis.

In a kinetic examination of the reaction of hydrazine with the four common nucleotides of RNA at different *p*H values and temperatures, we found, however, that it attacks both uridylic and cytidylic acids to such an extent that it is impossible to obtain a sufficiently specific degradation of either of them.

The work of H. Schuster on inactivation by hydroxylamine of biologically active nucleic acids turned our attention to this reagent, which has recently been used extensively as a mutagenic agent[8,9]. Preliminary investigations of the reaction kinetics in our laboratory soon proved the superiority of hydroxylamine to hydrazine for our purpose.

A salt-free aqueous solution of hydroxylamine in concentrations of 3–10 molar was incubated at different temperatures and *p*H values with the common nucleotides, nucleosides and corresponding bases. Reaction-rates were followed spectrophotometrically after electrophoretic separation of the reaction products. No reaction was found in the case of adenine and its derivatives, whereas those of guanine and thymine reacted only very slowly under our experimental conditions.

Both uracil and cytosine nucleotides and nucleosides are attacked, but the free bases react much more slowly. The dependence on *p*H of the reaction-rates of the groups differ appreciably. The *p*H optimum for the reaction with uridylic acid lies at *p*H 10·1, whereas that for cytidylic acid is 6·1. Fig. 1 gives the reaction-rates, as measured by percentage of

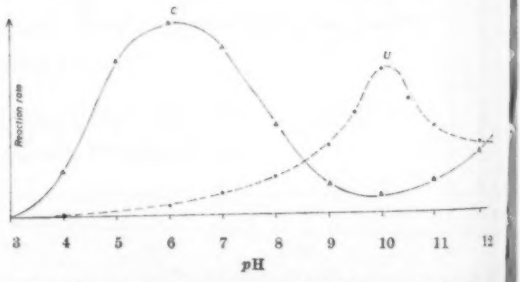

Fig. 1. Dependance of rate of reaction of 6 *M* aqueous hydroxylamine with 2′,3′-uridylic acid (U) and 2′,3′-cytidylic acid (C). Reaction-rate measured as percentage of decrease of extinction in 10 min. during the initial reaction phase

decrease of extinction during a 10-min. interval in the linear phase of the reaction, as a function of the *p*H.

This curve clearly indicates a possibility of degrading cytidylic acid or uridylic acid selectively by controlling the *p*H. This possibility is, however, limited at *p*H 10·0, where cytidylic acid still reacts to some extent. Furthermore, when applied to nucleic acids, this *p*H leads to a slow alkaline hydrolysis of the chain of about 1 per cent per hr. In order to investigate the possibility of increasing the specificity of the reaction, and to evaluate the extent to which RNase action would be modified, an investigation of the chemical nature of the reaction and the reaction products was conducted.

Reaction of Uridylic acid and Uridine with Hydroxylamine

The rate of the reaction is directly proportional to the concentration of hydroxylamine in the range of concentrations investigated. Under our conditions, the reaction kinetics corresponded to those of a pseudo first-order reaction. Products of the reaction were separated electrophoretically and consisted mainly of urea, ribose oxime or its phosphate and an unknown substance with strong ultra-violet absorption. Urea and ribose oxime were identified by comparing the chromatographic and electrophoretic behaviour with standards. The other product was identified as isoxazolone-5, a substance of which only derivatives were known hitherto. Its structure is based on the following data. The electrophoretic mobility and potentiometric titration curve indicate an acid with pK near 3·4. The substance is stable only in the anionic form and decomposes rapidly in acid to form a variety of products, among others a hydroxamic acid. The ultra-violet spectrum is in agreement with the proposed structure, with an absorption peak at 247 mμ and a specific extinction of $7{\cdot}9 \times 10^{-3}$ at *p*H 13·0. Catalytic hydrogenation with Raney nickel or platinum ($Pt[H_2]$) yields β-alanine, ruling out the possible alternative configuration of isoxazolone-3, which would have given β-hydroxypropionic acid amide. Finally, the substance was synthesized from both the sodium salt and the oxime of formyl acetic ester and from propiolic acid ethyl ester by reaction with hydroxylamine (Fig. 2).

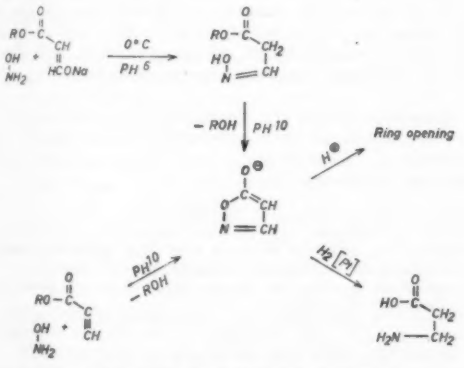

Fig. 2. Synthesis and reactions of isoxazolone-5

The synthetic product was identical in electrophoretic behaviour to the uridylic acid derivative; it also yielded β-alanine on hydrogenation and the acid breakdown products from both were chromatographically identical in four different solvent systems.

The reaction can be formulated in terms of a nucleophylic attack by the hydroxylamine nitrogen on the electrophylic C(4), or by the oxygen on C(6), followed by a cyclization and the formation of isoxazolone-5 and elimination of urea riboside or its phosphate from which the urea is displaced by another molecule of hydroxylamine (Fig. 3).

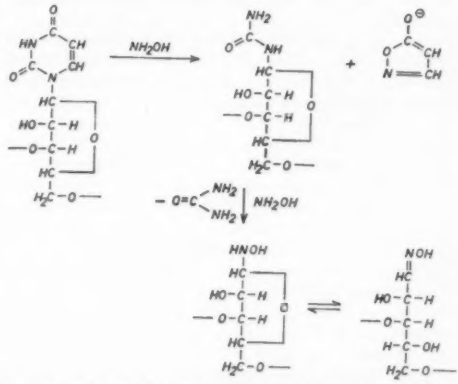

Fig. 3. Reaction of hydroxylamine with uridine and uridylic acid

The substitution of the N(3) atom in nucleosides and nucleotides would weaken its basic character, thereby increasing the susceptibility of C(4) or (6) to nucleophilic attack. This would explain why uracil reacts only very slowly. The slow-down of the reaction above *p*H 10·0 is probably due to the dissociation of the first hydroxyl group of uracil at *p*H 9·5. In the case of isocytosine, where the basicity of the ring is decreased, the rate of reaction with hydroxylamine is accelerated to such an extent that the free base reacts comparably to uridylic acid, also yielding isoxazolone-5.

Reaction of Cytidylic acid and Cytidine with Hydroxylamine

The ultra-violet extinction of cytidylic acid is obliterated within 90 min. by treatment with hydroxylamine at the optimum *p*H of 6·0. Electrophoretic separation of this mixture at *p*H 3·5, however, yields mainly a strong ultra-violet absorbing band with a mobility between that of guanylic and uridylic acids. This substance was isolated by preparative electrophoresis on a 'Sephadex' block, purified on a 'Dowex-50' column, crystallized and identified as uridylic acid-6-oxime. The ultra-violet spectrum corresponds to that obtained by Fox and Bendich[10], being similar to that of cytidylic acid in the acid region with a peak at 278 mμ, which disappears at neutral *p*H. Electrophoretic mobility indicates a compound with a negative charge between that of uridylic and cytidylic acids at *p*H 3·5 and containing ribose and phosphate. On hydrolysis in 1 *N* hydrochloric acid for 1 hr. at 100° C., the substance yields uridylic acid, which was identified by electrophoretic and chromatographic comparison with a reference, as well as hydroxylamine. The uridylic acid 6-oxime was found to be relatively stable, being hydrolysed only very slowly in acid or alkali at 37° C.

The initial disappearance of the ultra-violet extinction at *p*H 6·0 indicated that the uridylic acid 6-oxime was a secondary product and that some intermediate

is involved. This intermediate was partially purified by electrophoresis at *p*H 6·0 and could be converted quantitatively into uridylic acid 6-oxime by incubating in *N*/10 hydrochloric acid in a boiling water-bath for a few minutes. Bautz and Freese[11] also recently observed this reappearance of the ultra-violet extinction after hydroxylamine treatment of cytidylic acid, but thought it was due to reconversion to cytidylic acid itself.

In the presence of an excess of hydroxylamine the intermediate is degraded at *p*H values above neutral, possibly by a similar mechanism to that of uridylic acid breakdown, because two identical end products giving a positive hydroxamic acid reaction with ferric chloride reagent are found. No equivalent heterocyclic intermediate could, however, be isolated. An analysis of the intermediate yielded a nitrogen/phosphorus ratio of 3·93 : 1. An assumption that hydroxylamine was added to the 1,6 double bond of cytidylic acid yielding 6-hydroximino-1,6-dihydrocytidylic acid, could explain the spectrophotometric observations and was apparently supported by the demonstration of ammonia, later found to be a contaminant, in the acid degradation product. It was not, however, in accordance with the Pullmans' calculation that the C(4) would be the most reactive centre in this reaction[12], and neither could it explain the anomaly between the reaction of hydroxylamine with cytidine monophosphate and with RNA (see below).

Evidence was obtained by Brown[13] that two molecules of hydroxylamine take part in the reaction and that the 4,5 double bond disappears. This indicated a two-stage reaction with an initial addition of hydroxylamine to the double bond followed by substitution of the amino group by a second molecule of hydroxylamine. Further supporting evidence for this hypothesis was obtained in our laboratory by demonstrating electrophoretically the formation of ammonia in the reaction yielding the intermediate, and of free hydroxylamine during the conversion of intermediate to oxime. The reactions described are summarized in Fig. 4.

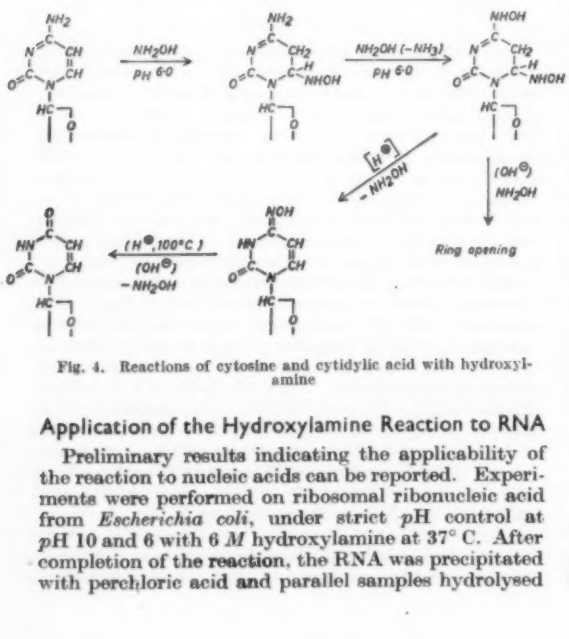

Fig. 4. Reactions of cytosine and cytidylic acid with hydroxylamine

Application of the Hydroxylamine Reaction to RNA

Preliminary results indicating the applicability of the reaction to nucleic acids can be reported. Experiments were performed on ribosomal ribonucleic acid from *Escherichia coli*, under strict *p*H control at *p*H 10 and 6 with 6 *M* hydroxylamine at 37° C. After completion of the reaction, the RNA was precipitated with perchloric acid and parallel samples hydrolysed in alkali and pancreatic ribonuclease respectively. Mono- and oligo-nucleotides were separated electrophoretically on 'Sephadex *G* 75'.

At both *p*H values the overall reaction-rate was slower with RNA than with mononucleotides, probably due to the secondary structure of the RNA. The specificity remains the same, mainly the uridylic acid residue being degraded at *p*H 10, although a certain amount of cytidylic acid breakdown also occurs. In the RNase hydrolysate after *p*H 10 treatment, virtually all the uracil monophosphate disappears. That RNase does not hydrolyse the chain at the sites where uracil has been removed is more conclusively shown by the marked decrease of cytidine monophosphate as well as of oligonucleotides with electrophoretic mobilities near those with uridylic acid end-groups in untreated RNA hydrolysates. During incubation at *p*H 10 for 3 hr., about 5 per cent of the RNA becomes soluble in acid, indicating non-specific alkaline hydrolysis and possibly some hydrolysis caused by a β-elimination reaction.

At *p*H 6·0 essentially comparable results were obtained, oligonucleotides with cytidine monophosphate end-groups diminishing markedly and cytidine monophosphate disappearing from the hydrolysate. The alkaline hydrolysate of the same material showed no decrease in cytidine monophosphate content, however. This can be explained by assuming that secondary structure bonding permits only the addition of the first hydroxylamine molecule to the 4,5 double bond of the uracil residue, thereby changing its aromatic character and inhibiting the RNase action. The first-stage intermediate thus formed is then reconverted to cytidylic acid during the alkaline incubation. This reconversion could not be obtained with mononucleotides, indicating that the second stage of the reaction, the substitution at C(6), is irreversible.

The results of this investigation indicate that it is possible to modify nucleic acids by chemical means in such a way that a defined enzymic partial hydrolysis different from, and of higher specificity than, that obtained without the treatment becomes feasible.

The foundation for the development of a chemical partial hydrolysis procedure on the β-elimination principle has also been laid. This could be achieved, for example, by adding a suitable amine during the reaction of nucleic acid and hydroxylamine at *p*H 10.

The specificity of the reaction, especially at *p*H 10·0, must still be improved. The investigation of the reaction of hydroxylamine with nucleic acid, and the possible ways of effecting partial hydrolysis of the product, has only started and is being continued.

We acknowledge the support and interest of Prof. A. Butenandt, in whose laboratory this work was performed. Thanks are due to Dr. H. Schuster for stimulating discussions.

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FERROMAGNETIC INCLUSIONS IN NUCLEIC ACID SAMPLES

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BROAD, occasionally very intense, magnetic resonance absorption signals have been observed in a wide variety of preparations of nucleic acids[1–4]. The origin of these signals has not been specified, though it has been inferred that large numbers of unpaired electron spins (as many as 10^{21} spins/gm. in some cases) are inherently present in these and certain other organic structures of high molecular weight. A critical point in these arguments has been the purported absence of inorganic, magnetic impurities in quantities sufficient to explain the observed resonance intensities.

Recently it has been found that samples yielding electron-spin resonance spectra similar to certain of those reported earlier exhibit *ferromagnetic* rather than *paramagnetic* behaviour[5]. The possibility that the resonance depends critically on the existence of a polymeric organic structure has been disproved since denaturation of deoxyribonucleic acid (DNA)[4] or even heating well above the charring point does not destroy the electron-spin resonance signal[6]. Furthermore, trace quantities of iron, the dominant iron-group impurity, have been found in amounts sufficient to explain the observed magnetism if it is assumed that the magnetic ions are present as concentrated ferromagnetic aggregates rather than as disperse paramagnetic centres[5,6]. We now wish to summarize a series of subsequent experiments which confirm this hypothesis and which permit a good guess as to the specific iron compound involved as well as its origin.

The generality of the phenomenon has been established by examination of a large number of nucleic acid samples as indicated by Table 1. Though a variety of signal shapes have been reported we are here concerned only with the (in our experience) dominant and well-defined absorption line characterized at room temperature by a maximum at $g = 0{\cdot}71444\ f/H = 2{\cdot}15\text{–}2{\cdot}25$ (f is the frequency of observation in Mc./s.; H, the field for maximum absorption in gauss) and a width between points of largest slope of $\Delta H \simeq 1{,}000\text{–}1{,}500$ gauss. Though both g and ΔH increase appreciably in magnitude as the temperature is decreased the intensity of the resonance remains constant within experimental error from 300° K. down to 20° K. at least.

The ferromagnetic nature of the phenomenon has been confirmed in the case of the three most strongly magnetic samples by demonstration of remanent moments and coercive forces[5]. The static magnetic moments of these samples also proved independent of applied field and ambient temperature over wide ranges as expected. Quantitative comparisons between the electron-spin resonance intensities and the static moments gave agreement within the experimental errors. Such comparisons for two DNA samples are shown in Table 2.

The number of unpaired spins responsible for an observed electron-spin resonance signal depends on whether the spins are paramagnetic or ferromagnetic. The ratio of these spin concentrations is:

$$\frac{N_{\text{para}}}{N_{\text{ferro}}} = \frac{3kT}{g\beta H_0}\,\frac{S'}{S(S+1)}$$

in which k is the Boltzmann constant, T the temperature, g the observed g-factor, β the Bohr magneton, H_0 the magnetic field, S' the electron spin in the ferromagnetic state and S the spin in the paramagnetic state. For $S' = 2$ and $S = 1/2$ this ratio is slightly larger than 4,000 for experiments performed at 300° K. with 11·7-kMc./s. microwaves. The intensity of the nucleic acid absorption signal has been compared with that due to a known concentration of paramagnetic ions in a diamagnetic host (1 per cent Ni^{2+} in $KMgF_3$) in order to calculate the number of paramagnetic spins ($S = 1/2$) required to produce an equivalent electron-spin resonance absorption. The comparison can, of course, only be made at a particular temperature, 300° K. being chosen in the present work. A rough indication of these equivalent spin densities is given in Table 1. More exact numbers are shown for the two strongly magnetic samples in Table 2, as are ferromagnetic spin densities ($S' = 2$) calculated on the basis of the formula given above.

Identification of iron as the most likely source of the observed ferromagnetism had been established by X-ray fluorescence analysis[5] (see also ref. 4). In Table 2 we present the results of a quantitative analysis of the amount of iron in these samples which was not removed by treatment with versene. The concentration of iron is much larger than N_{ferro}. Lack of numerical agreement between the two sets of numbers is not an argument against inorganic iron compounds as the source of the ferromagnetism since the excess iron can be accounted for in a number of ways, some of which are mentioned later.

Table 1. SAMPLES EXAMINED WITH ELECTRON-SPIN RESONANCE TECHNIQUES

The intensity refers to a specific absorption line described in the text. The criteria, strong, medium, weak, and very weak, correspond crudely to more than 10^{20}, 10^{20}–10^{19}, 10^{19}–10^{18}, less than 10^{18} equivalent paramagnetic electron spins ($S = 1/2$)/gm. at room temperature

Sample	Source	ESR intensity
Herring sperm DNA	Nutritional Biochemical	strong
Salmon sperm DNA	California Corp. for Bio. Res.	medium
Calf thymus DNA ΣI	Sigma Chemical Co.	weak
,, ,, ,, ΣII	,, ,, ,,	strong
,, ,, ,, ΣII′	,, ,, ,,	strong
,, ,, ,, *W*593	Worthington Biochem.	weak
,, ,, ,, *W*594	,, ,,	weak
,, ,, ,, *W*595	,, ,,	very weak
,, ,, ,, *SK*	Dr. L. Cavalieri, Sloan-Kettering Institute	weak
Tetranucleotide DNA	Prof. A. Rich, M.I.T.	medium
Yeast RNA M	Mann Biochemical	weak
,, ,, S	Schwartz Biochemical	weak
Aerobacter aerogenes RNA	Prof. A. Rich, M.I.T.	weak
Bacteriophage (*T*2)	Prof. E. Lennox, N.Y.U. Bellevue	weak*
Salmon sperm heads	Dr. N. Simmons, U.C.L.A.	weak*

* See text for qualifications.

Table 2. COMPARISON OF SPIN RESONANCE, STATIC MAGNETIC MOMENT AND IRON ASSAYS OF TWO STRONGLY FERROMAGNETIC SAMPLES OF DNA

All listed quantities are per gm. of nominally dry material

Sample method	Herring sperm DNA	Calf thymus DNA ΣII′
Electron-spin resonance	9×10^{20} para $S = 1/2$ $2{\cdot}2 \times 10^{17}$ ferro $S' = 2$	16×10^{20} para $S = 1/2$ $3{\cdot}8 \times 10^{17}$ ferro $S = 2$
Static magnetic moment	14×10^{20} para $S = 1/2$ $3{\cdot}4 \times 10^{17}$ ferro $S = 2$	6×10^{20} para $S = 1/2$ $1{\cdot}4 \times 10^{17}$ ferro $S = 2$
Bound iron	0·048 weight per cent 5×10^{18} atoms	0·049 weight per cent 5×10^{18} atoms

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Since the existence of the iron in a separate, probably inorganic phase was suspected, several samples were examined by electron microscopy. Dense platelets were found rather randomly embedded in groups of DNA molecules (Fig. 1) (I. Isenberg has also observed such inorganic inclusions in protamine nucleate by electron microscopy—private communication). The diffraction pattern of the platelets definitely identifies them as single crystals of inorganic compounds (Fig. 2) though no chemical identification was possible from these discrete Laue patterns taken in arbitrary crystal directions. The relative number densities and average sizes of these inclusions (~ 1000 Å. maximum linear dimension) correlates quite well with the relative levels of magnetism found. It has not been possible to remove the platelets by simple filtration of water solutions, filtration after ultrasonic agitation or centrifugation. A variety of quite large inorganic crystallites were brought down by centrifugation and filtration, some of these even proving quite strongly magnetic; however, the intensity of the electron-spin resonance signal in the supernatant DNA and the occluded platelet count were not appreciably changed. It appears that the embedded inorganic particles are tightly bound to the surrounding nucleic acid molecules which serve to protect the particles from chemical attack and drag the nucleic acid 'shroud' with them when mechanical separation is attempted.

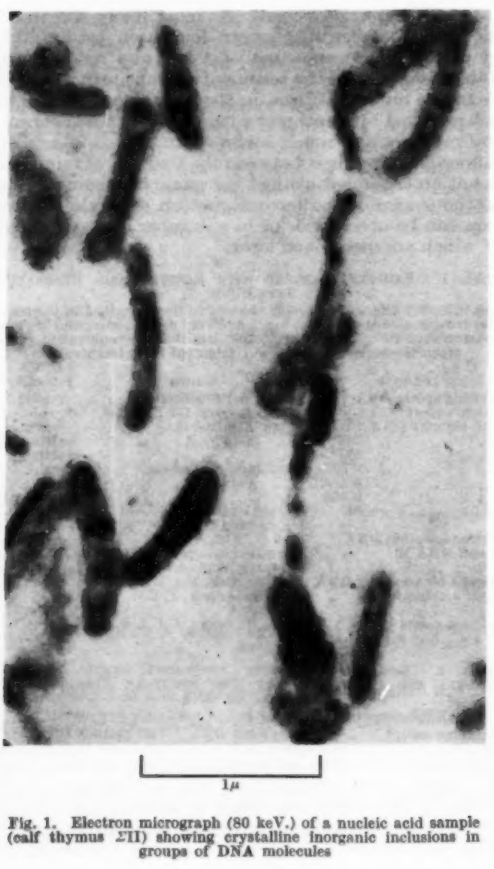

Fig. 1. Electron micrograph (80 keV.) of a nucleic acid sample (calf thymus ΣII) showing crystalline inorganic inclusions in groups of DNA molecules

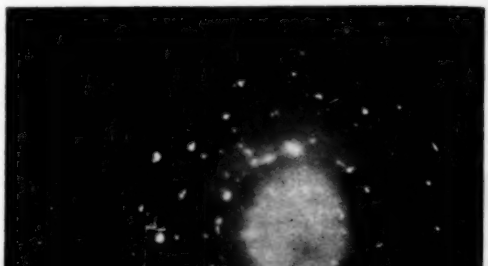

Fig. 2. Selected area electron diffraction pattern (80 keV.) from a group of crystalline inclusions similar to those shown in Fig. 1

Failure to identify the chemical composition of the occluded platelets by electron diffraction and to separate it from its host DNA for chemical analysis led to attempts at magnetic identification. The electron-spin resonance signal of a DNA sample (calf thymus ΣII′) has been observed as the microwave cavity was heated with a nitrogen gas atmosphere present. The spectrum found at 900° K. is quite similar to that seen at 300° K., but the line intensity decreases and the line shape becomes complex at somewhat higher temperatures though it returns to its usual shape and intensity on lowering the temperature. At these high temperatures the DNA is completely charred, once again emphasizing the lack of correlation between the ferromagnetic resonance and the molecular structure of DNA. (A sharp paramagnetic free-radical resonance is observed at $g = 2{\cdot}00$ after the sample has charred appreciably.) Since the ferromagnetic resonance is still visible at 900° K. we can eliminate Fe_3O_4 as its origin since its Curie temperature is 855° K. While αFe_2O_3 with a Curie temperature of 950° K. appears possible, it must be eliminated since it is a parasitic ferromagnetic material the magnetization of which is too small to account for the observed signal. Also the resonance of αFe_2O_3 disappears suddenly near 260° K. whereas that of our nucleic acid samples does not[7]. This might, however, account for the disappearance of resonance reported by Bliumenfeld[1]. (Another alternative in this regard would be Fe_3O_4 since its resonance is reported to broaden quite rapidly below 115° K. (ref. 8).) At present we are unable to suggest a specific iron compound the properties of which exactly parallel the behaviour of our nucleic acid samples. However, we do find a close parallelism in a preparation of colloidal magnetic particles produced by coprecipitation of Fe^{2+} and Fe^{3+} ions from solution[9]. These particles are of nominal composition Fe_3O_4, but evidently differ significantly from macroscopic crystals of that material. It would appear that this oxide-hydroxide of iron is the form responsible for the greater part of the resonances observed in nucleic acid preparations.

The rather low moment per iron atom found in the nucleic acid samples may be due to a number of causes: partial compensation of one magnetic sub-

lattice by another (ferrimagnetism), lack of alignment of magnetic moments near the surface of particles due to strong local anistropy, formation of additional weakly magnetic phases, for example, αFe_2O_3, iron hydroxide, isolated ions.

The next question is whether or not the iron ions which are commonly found as trace impurities in nucleic acid samples exist in a ferromagnetic form *in vivo*. We examined the resonance spectrum of salmon sperm heads which had been prepared, dialysed extensively and treated with versene by Dr. N. Simmons. When they were first observed no broad electron-spin resonance was observed, in spite of the fact that Simmons's analysis showed a large concentration of iron. However, after storage at ~4° C. for several months, the electron-spin resonance spectrum was examined again and a weak ferromagnetic resonance was observed. In a similar fashion we observed the electron-spin resonance spectrum of a freshly prepared sample of bacteriophage *E. coli T*-2 which was kindly given to us by Prof. E. Lennox. At first the dried bacteriophage showed several sharp resonance lines, characteristic of paramagnetic impurities. However, after several months it too showed a weak ferromagnetic resonance which was not discernible at first.

In our opinion these observations are best explained by the following hypothesis: the iron found in nucleoproteins is present in the living cells though not in the concentrated ferromagnetic form. The latter, an oxide-hydroxide perhaps approximating Fe_3O_4 in composition, may be precipitated during extraction of nucleic acids or may even form quite slowly in intact but dried organisms. The observed clumping of nucleic acid molecules about the inorganic platelets may be evidence for nucleation of the colloidal precipitation at charged sites, for example, the phosphate side groups. That the iron and other trace metals found in nucleic acid samples are indeed present in the biological source is assured by the work of Wacker and Vallee[10].

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VARIATIONS IN THE NUCLEAR DEOXYRIBONUCLEIC ACID CONTENT IN THE ADRENAL CORTEX OF THE FEMALE WHITE RAT DURING THE ŒSTRUAL CYCLE

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USING the cytophotometric technique for determination of the mean nuclear deoxyribonucleic acid (DNA) content as a test of cellular activity, one of us[1] investigated the function of the different layers of the adrenal cortex. Arguments in favour of the opinion of Deane and Greep[2] were provided. According to these authors the zona fasciculata is the glucocorticoid-producing layer, which function is dependent on the adrenocorticotrophic hormone secretion of the adenohypophysis; the zona glomerulosa is the mineralo-corticoid-producing layer and independent of the hypophysis. The zona fasciculata interna and the zona reticularis are considered to be reserve layers. In the present investigations we have investigated the nuclear DNA content in the adrenal cortex of the female white rat during the different stages of the œstrual cycle. It has already been shown by several authors[3-6] that the adrenal weight increases during œstrus. Hunt[7] has observed variations of the mitotic activity in the zona glomerulosa during the œstrual cycle.

In a series of female white rats the œstrual cycle was determined by observing vaginal smears during two weeks. After this period 5 animals in dicœstrus, 4 in procœstrus and 5 in œstrus were selected and killed. The adrenals were then fixed, dehydrated, embedded, sectioned and stained by the Feulgen reaction. The DNA content of about 50 individual nuclei in every layer of the cortex was determined by means of the cytophotometric technique of Lison[8]. Table 1 shows a survey of the pooled results.

The distribution of the nuclear DNA in the different layers during dicœstrus is very similar to that observed in the male cortex. This content is indeed significantly lower in the zona fasciculata interna and reticularis than in the zona fasciculata externa ($P < 0.001$). In the zona glomerulosa, however, this content is 6 per cent lower than in the fasciculata externa ($P < 0.001$). In the male rate the DNA content is generally the same in these two layers. In a few instances only, a lower content was observed in the zona glomerulosa[1]. In procœstrus the general distribution is preserved, but in every layer the DNA content is decreased. This decrease is not,

Table 1

		$\bar{x}$	$\log\bar{x}$	s	$s_{\log\bar{x}}$	n
Dicœstrus	*G.*	1,093	3·0387	0·0841	0·0053	246
	F.E.	1,159	3·0643	0·0622	0·0039	245
	F.I.	1,048	3·0207	0·0614	0·0039	247
	R.	1,082	3·0344	0·0684	0·0043	246
Procœstrus	*G.*	982	2,9920	0·0584	0·0041	195
	F.E.	1,079	3·0331	0·0687	0·0048	198
	F.I.	1,006	3·0027	0·0572	0·0040	197
	R.	978	2·9904	0·0631	0·0044	200
Œstrus	*G.*	1,272	3·1045	0·0681	0·0043	243
	F.E.	1,159	3·0641	0·0618	0·0039	244
	F.I.	1,197	3·0781	0·0503	0·0032	247
	R.	1,226	3·0955	0·0542	0·0034	247

G., zona glomerulosa; *F.E.*, zona fasciculata externa; *F.I.*, zona fasciculata interna; *R.*, zona reticularis.

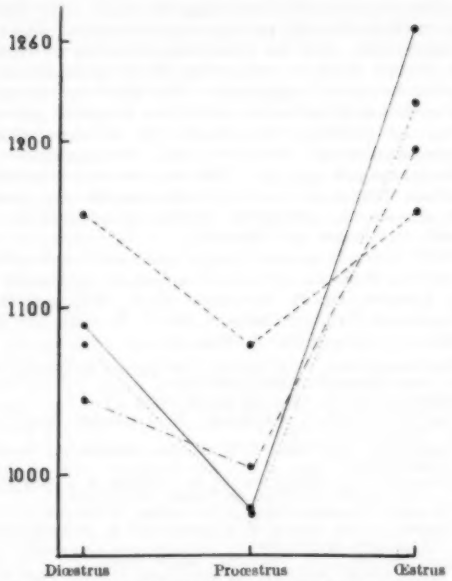

Fig. 1. Variations in the nuclear DNA content in the different layers of the adrenal cortex during the œstrual cycle
– – –, zona fasciculata externa; ——, zona glomerulosa;
. . . ., zona reticularis; —·—·, zona fasciculata interna

however, significant in the zona fasciculata interna. In œstrus the nuclear DNA distribution is strikingly changed. The differences between the zona fasciculata externa and the other layers are reversed. In comparison with the proœstrus the DNA content in the zona glomerulosa is increased by 29 per cent and exceeds the zona fasciculata by 9 per cent. The DNA content of the zona fasciculata externa returns to the value observed during diœstrus. The DNA content of the zona fasciculata interna is increased by 19 per cent and by 27 per cent in the zona reticularis. Fig. 1 summarizes these variations during the œstrual cycle.

The decreased DNA content in the four layers during proœstrus suggests a general inhibition of cell activity during this period. We have not found any biochemical and physiological observation in the literature until now which could support this conclusion. The most prominent variations are found, however, during œstrus. The increased DNA content in the zona fasciculata interna and reticularis suggests a stimulation of the corticosterone secretion. This result fits in with the already observed increase in adrenal weight[2–5] during œstrus and after œstrogen treatment[6] and with the increase in nuclear DNA in the zona reticularis of the male rat after œstrogen administration[1]; it is also in agreement with the biochemical analysis of ketosteroid excretion during the œstral cycle[8]. The changes of the nuclear DNA content develop within the very short time of one day. Very striking, also, is the increase of 29 per cent observed in the zona glomerulosa during œstrus. This would indicate a stimulation of the aldosterone secretion. Hunt[7] has already observed an increased mitotic activity in this layer. Only a few biochemical data are available at present to explain this observation. Gormall, Robertus and Laidlow[10] found a rise of aldosterone excretion after œstrogen treatment in one patient. Our results fit in with earlier observations on the influence of stilbœstrol on the cortex of guinea pig and hamster[5].

These observations are once more in contradiction to the general agreement of the constancy of nuclear DNA and show that the variations of the DNA content may develop within a very short time in correlation with cell-activity.

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EFFECT OF PLANT GROWTH COMPOUNDS ON UPTAKE OF AMINO-ACIDS BY PLANT ROOTS

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DURING an examination of the uptake of amino-acids by plant roots[1], it was noted that the herbicide 2,4-dichlorophenoxyacetic acid affected the amount of amino-acid taken up. Since other workers[2–4] have reported effects by indole-3-acetic acid or 2,4-dichlorophenoxyacetic acid on uptake of solute it was decided to examine this effect further.

Seeds sterilized with a dilute hypochlorite solution were germinated and grown in the dark at 25° under sterile conditions in Petri dishes containing filter paper moistened with a dilute salt solution. After 5 days the roots were excised, washed, and blotted dry for weighing into 50-ml. Erlenmeyer flasks or in Warburg vessels. The flasks contained 0·02 *M* sodium phosphate solution, *p*H 5·2, 2 m*M* radioactive amino-acid and any additions as required. The flasks were incubated in air for 2 hr. at 25°, the roots washed free of medium and extracted with 80 per cent ethanol. The radioactivity released from the roots was determined by drying an aliquot on an aluminium planchet and counting under a thin-window gas-flow counter. Full details of the methods used are reported elsewhere[1].

The effect on glycine transport into mustard roots by several plant growth substances is shown in Table 1. In this experiment the roots were incubated for 45 min. in 5×10^{-5} *M* solutions of the hormones before adding the labelled glycine.

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Table 1. Effect of Plant Growth Substances on Uptake of Glycine by Mustard Roots

Growth substance	Relative glycine uptake (percentage of control)
Indole-3-acetic acid	84
Indole-3-butyric acid	56
Indole-3-propionic acid	57
Ethylindoleacetate	94
Gibberellic acid	99
Maleic hydrazide	88
Furyl acrylic acid	8
2,4-Dichlorophenoxyacetic acid	39
2,4,5-Trichlorophenoxypropionic acid	16
2,4,5-Trichlorophenoxyacetic acid	29
Naphthalene acetic acid	55
β-Naphthoxyacetic acid	55
Methylnaphthaleneacetate	61
Naphthalene acetamide	104

Roots were incubated in 0·02 *M* sodium phosphate solution, *p*H 5·2 with 2 m*M* glycine-1-^{14}C. Growth substances were added to give a final concentration of 5×10^{-4} *M*, 45 min. prior to adding the radioactive glycine.

The most effective inhibitor was furyl acrylic acid followed by the commonly used herbicides 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenoxypropionic acid. Indolebutyric, indolepropionic, naphthaleneacetic and β-naphthoxyacetic acids, and methylnaphthalene acetate all reduced uptake by 40–50 per cent. Indoleacetic acid, ethyl indoleacetate, gibberellic acid, maleic hydrazide and naphthalene acetamide had little or no effect.

The inhibition by 2,4-dichlorophenoxyacetic acid was not confined to the uptake of glycine by mustard roots. Under the same conditions as in Table 1, this herbicide reduced transport of methyl labelled L-methionine into mustard by 50 per cent and glycine transport into wheat roots by 40 per cent.

Different concentrations of 2,4-dichlorophenoxyacetic acid were tested for inhibitory action (Table 2). At concentrations of 10^{-5} *M* and less, there was either no effect or a slight stimulation of glycine transport and respiration. At 10^{-4} *M*, oxygen consumption was unaltered while glycine uptake was reduced by 26 per cent. At 2×10^{-4} *M*, oxygen uptake was lowered by 18 per cent and glycine transport by 52 per cent. At higher concentrations, both glycine and oxygen uptake were markedly reduced.

Table 2. Effect of Different Levels of 2,4-Dichlorophenoxyacetic Acid and 2,4-Dinitrophenol on Uptake of Glycine by Mustard Roots

Addition to medium	Concentration of addition (*M*)	Relative uptake (percentage of control) Glycine	Oxygen
	—	100	100
2,4-Dichlorophenoxyacetic acid	10^{-5}	18	18
	5×10^{-4}	27	38
	2×10^{-4}	48	82
	10^{-4}	74	100
	5×10^{-6}	109	109
	10^{-6}	97	118
	10^{-7}	99	110
2,4-Dinitrophenol	10^{-4}	10	20
	10^{-5}	43	124
	10^{-6}	97	94

Roots were incubated in Warburg flasks in 0·02 *M* sodium phosphate *p*H 5·2, 2 m*M* glycine-1-^{14}C and 2,4-dichlorophenoxyacetic acid or 2,4-dinitrophenol at the concentrations given above. The flasks were shaken for 2 hr. at 25°, the gas phase being air.

These results can be compared with those found using 2,4-dinitrophenol, where glycine transport was lowered despite a stimulation in respiration. This suggests the possibility that the actions of 2,4-dichlorophenoxyacetic acid and 2,4-dinitrophenol are similar and perhaps due to effects on oxidative phosphorylation.

Preliminary experiments with the incorporation of phosphate-32 into root nucleotides provide supporting evidence for an effect on oxidative phosphorylation by 2,4-dichlorophenoxyacetic acid as reported by Switzer[5] and Khubutiya[6].

It is reasonable to conclude that the reduction of amino-acid transport into plant roots caused by 2,4-dichlorophenoxyacetic acid is probably due to reduced levels of adenosine triphosphate, since uptake of amino-acid is known to be an energy-requiring process[1].

This work was supported by a grant from the National Science Foundation. I wish to thank Prof. J. H. Quastel for suggesting the topic and for his guidance.

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TESTS FOR LIVER DYSFUNCTION IN DOGS

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THE reason for undertaking this investigation was to try to select a few simple biochemical techniques which would enable the veterinary surgeon to gain useful information about the state of the patient's liver without having to resort to laparotomy or biopsy.

The pattern of the work had to be governed by the material available, which was provided, for the main part, by the Beaumont Animals Hospital. It was not considered practical to undertake any test which necessitated even the temporary hospitalization of the patient, or the preliminary intravenous injection of any substance (for example, sulphobromphthalein). Therefore, tests had to be developed which could be carried out on a single blood sample.

Serum Alkaline Phosphatase. Estimations of serum alkaline phosphatase were carried out on 463 samples, and the normal value was found to be 8 King Armstrong (K.A.) units. In dogs, a species in which extrahepatic obstructive jaundice would appear to be a less common clinical entity than in humans, very high values were given by hepatocellular jaundice, for example, a value of 120 K.A. units was found in a case of leptospiral jaundice; and it is an interesting speculation that the pathology of the hepatic cell of the dog may differ from that of man, possibly showing a greater tendency to undergo cloudy swelling and thus to produce intrahepatic obstruction.

Raised serum alkaline phosphatase values were also found in young animals undergoing active growth of bone tissue, and in conditions in which there was an interference with calcium and phosphorus metabolism; that is, bone lesions (9–90 K.A. units), and

some cases of nephritis (9–60 K.A. units), cystic calculi (10–40 K.A. units) and enteritis (9–100 K.A. units). My experience of this test showed that 95 per cent of dogs with definite liver involvement gave positive results, and so did 75 per cent of dogs with suspected liver involvement. It also indicated the main disadvantage of the test, which is that high values are given by conditions which do not involve the liver (*vide supra*).

Serum Transaminases. Serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase.

Normal values were calculated from estimations carried out on the sera of 11 young healthy dogs, and were taken as follows: serum glutamic pyruvic transaminase less than 20 Sigma Frankel units, serum glutamic oxaloacetic transaminase less than 30 Sigma Frankel units. These values were comparable with those of Cornelius, Bishop, Switzer and Rhode[1], and Malherbe[2]. The results of estimations on more than 70 different clinical cases indicated that increases of transaminase activity, especially serum glutamic pyruvic transaminase (Hoe[3]), are reliable indications of early liver cell damage, before it is indicated by other tests. For example, in four puppies which had been inoculated with the virus of hepatitis contagiosa canis, considerable increases in serum glutamic pyruvic transaminase could be seen 5 days after inoculation while other liver function tests remained normal. Serum glutamic oxaloacetic transminase was also raised, but not generally as significantly.

Total Serum Cholesterol. Total serum cholesterol values were calculated for two groups of normal dogs, household and kennel. Normal values were found to be: household dogs, 258·0 ± 36·3 mgm./100 ml.; kennel dogs, 186 ± 13·9 mgm./100 ml. It was thought that these different values were probably caused by dietary differences, the average domestic pet living in the house receiving a diet considerably higher in cholesterol than that fed to most kennel dogs. In view of these findings, cholesterol values greater than 299 mgm./100 ml. were taken as raised.

The estimation of total serum cholesterol was carried out on 250 sera. Although raised values were found in only 57 per cent of jaundice cases, this estimation would appear to be of considerable use in the diagnosis of other diseases, including nephritis, most cases of which gave values in the 300–499 mgm./100 ml. range. Raised values were also encountered in all cases of diabetes mellitus (310–1,360 mgm./100 ml.), and also in two animals which afterwards developed the disease, but which were not showing glucosuria at the time the serum was first examined; presumably this indicates a general disturbance in fat and carbohydrate metabolism found in the pre-diabetic patient. Two cases of hypothyroidism were encountered, and one of these was particularly interesting. When presented at the clinic, this Alsatian puppy was 9 months old, measured 25 cm. high and weighed 3·6 kgm. A diagnosis of hypothyroidism was made when the total serum cholesterol value was found to be 700 mgm./100 ml. This value decreased to the normal level when thyroid therapy was instituted.

Serum Uric Acid. About 70 per cent of the 240 cases examined during this survey gave uric acid values less than 0·5 mgm./100 ml., and only 11 per cent were higher than 1 mgm./100 ml. If one compares the relative merits of serum alkaline phosphatase and uric acid estimations as tests of liver function it can be seen that uric acid is not such a good index as serum alkaline phosphatase. While 95 per cent of the cases with the highest serum alkaline phosphatase levels showed some liver involvement, the corresponding figure for uric acid was only 75 per cent; also, while all cases of jaundice gave raised serum alkaline phosphatase values 86 per cent showed increased serum uric acid. 46 per cent of the cases of nephritis examined lay within the range 0·5–0·99 mgm./100 ml. It was noticed during this work that high uric acid figures frequently appeared to carry a poor prognosis for the patient, and hence were found in most moribund animals.

Serum Bilirubin. This estimation was carried out on some 300 sera, but much difficulty was experienced in evolving a technique which was sufficiently sensitive to measure the very low concentrations of bilirubin found in the sera of non-jaundiced dogs. The most suitable method seemed to be that of Powell[4]. No attempt was made during this investigation to differentiate between direct and indirect bilirubin. Accurate measurement of the small quantities involved presented so much difficulty that it was not deemed worth while attempting the more complicated techniques which would have been necessary, especially as hæmolytic jaundice does not seem to be of much practical importance in dogs in the United Kingdom.

It was also noted that dogs showing bilirubinuria did not in many instances show bilirubinæmia. It has been stated[5] that about 20 per cent of apparently normal dogs excrete bilirubin in their urine. This finding has been confirmed by Wilkinson (personal communication, 1960).

Serum Proteins. Relative percentages of serum proteins for normal dogs were calculated after electrophoresis had been carried out on the sera of 26 young healthy dogs living under similar conditions. The method of Jencks, Jetton, and Durrum[6] was used and results were as follows: albumin, 57·2 ± 0·95; α_1-globulin, 6·2 ± 0·37; α_2-globulin, 9·7 ± 0·46; β_1-globulin, 8·8 ± 0·54; β_2-globulin, 9·0 ± 0·37; γ-globulin, 10·4 ± 0·55; albumin : globulin ratio, 0·76 ± 0·087. Electrophoresis was also carried out on the sera of 140 dogs suffering from various diseases. Albumin decreased in most of the diseases investigated and this change was reflected in the albumin globulin ratio. Changes in α_2-globulin appeared to be non-specific. β_1-Globulin showed a significant increase in most of the neoplasia cases, but was decreased in all cases of jaundice and the majority of diabetes mellitus cases. β_2-globulin was a variable factor in most of the conditions studied but was always raised in jaundice. γ-Globulin was raised in jaundice and cirrhosis, but decreased in more than 90 per cent of the cases of diabetes mellitus, and it was felt that this was of some significance in view of the fact that such poor resistance to infection is shown by most human diabetic patients. Cirrhosis gave a characteristic electrophoresis diagram with poor resolution in the β- and γ-bands.

During this part of the investigation an attempt was made to establish a relationship between the results of the Kunkel turbidity test and the relative percentage of the γ-globulin as determined by electrophoresis. There was a significant correlation between the two factors, but the scatter about the regression line was too great to permit an accurate evaluation of γ-globulin by this method. It seems, however, that the Kunkel test can be used semi-quantitatively and this is in general agreement with the findings of most workers in human medicine.

The Takata Ara test appeared to be less specific than the Kunkel, and gave positive turbidities and flocculations for increases in any of the globulin fractions. From my work on these tests it appears that in the absence of a positive Kunkel, a positive reaction with the Takata Ara may indicate an increase in the globulin fractions other than the γ-globulin.

The most useful tests investigated seem to be serum alkaline phosphatase, the transaminases and electrophoresis, all of which can be performed on a a total of 0·9 ml. of serum. When it is possible these tests should be supplemented by the estimation of serum cholesterol, which can give useful information in cases of nephritis, pre-diabetes and in hypothyroidism.

The estimation of serum alkaline phosphatase has been shown to be a useful index of liver function, and when used with discrimination it will yield more reliable information than that given by serum bilirubin estimations, except in the case of hæmolytic jaundice. The transaminase tests (especially serum glutæmic pyruvic transaminase) play an important part in the diagnosis of active liver cell damage and necrosis before any abnormality is shown by any other liver function test. Electrophoresis is of considerable value, especially in the diagnosis of cirrhosis.

Practical details of this work are published elsewhere (Hoe and Harvey[7,8]).

I wish to thank Dr. D. G. Harvey for advice and help throughout this work, also Mr. G. C. Knight and my other colleagues in the Departments of Medicine and Surgery for the provision of clinical material. I would like to express the sincere gratitude I feel to the late Mr. A. B. MacIntyre, whose kindness and inspiration did much to further the work. I am indebted to the Trustees of the Clement Stephenson Fund for a scholarship.

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CHANGES OF BLOOD VOLUME IN THE PRE-HIBERNATING AND DEEP-HIBERNATING HEDGEHOG

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In hibernating animals the blood volume is decreased during the hibernation sleep. Although everyone who has been working on the physiology of hibernation is familiar with this decrease in blood volume, by simple observations, only scanty data on the blood volume of these animals appear in the literature[1,2].

In this Laboratory we have measured the blood volume of the common European hedgehog, *Erinaceus europaeus*, L. at normal body temperature (about 35° C.) as well as at low temperatures (about 5° C.). The measurements were made by way of the ordinary dilution method using Evans blue. The injections were mostly given into the inferior vena cava, and the samples mostly taken from this vessel.

By this method we found a marked decrease in blood volume in the hibernating hedgehog. In the 'summer animals' we found the blood volume to be about 8 per cent of the animals' weight, while in the sleeping 'winter animals' the blood volume was found to be about 2–3 per cent of weight (Fig. 1).

The question is raised as to how this decrease takes place. To find the answer to this question several analyses are made on the normal blood constituents of the animals, in different stages of hibernation and in the awakened state. Further, the weight of the spleen is measured in the same periods when looking for any connexion between blood volume and the well-known function of the spleen to accumulate erythrocytes. Finally, the most common constituents of the urine are measured in order to see if dehydration plays any part in the regulation of blood volume.

In this discussion it is important to know what we mean by the term 'different stages of hibernation'. As the term is being used by me it signifies three different periods of hibernation: the pre-hibernating period, the deep-hibernating period, and the period of arousal.

In the pre-hibernating period the body temperature is altering from the normal level (about 35° C.) to hypothermal levels (about 5° C.) and vice versa, in about 24 hr. Such variations in body temperature may take place about five to ten times during the period. The length of this period is about two months, in our animal room taking place in October–November. The same tendency of variation in body temperature in hibernating hedgehogs has been reported previously[3].

In the deep-hiberating period (from about December to about March) the body temperature is rather constant, at about that of the room. In the period of arousal the same variations in body temperature as those taking place in the pre-hibernating period, are never observed in this Laboratory. The end of

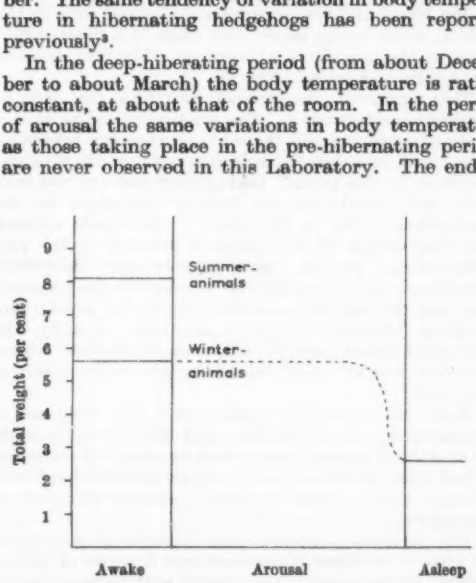

Fig. 1. Blood volume—measured as 'Evans blue volume'—of hedgehogs awake, during induced arousal, and in deep hibernation

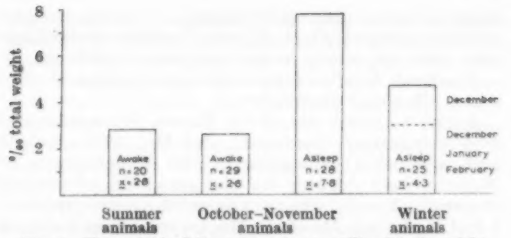

Fig. 2. The weight of the spleens in pro mille of body-weight, from hedgehogs in the summer, autumn and winter months
n No. of animals; x̄, mean value of weights. The diagram representing the spleen weights of the winter animals is divided into two parts by a stippled line. The lower part gives the situation of the spleen weights from animals in deep hibernation, while the upper part gives the corresponding data from animals entering the deep hibernating period

this period is about April, and from that time onwards the animals are named 'summer animals'.

I have measured a great number of spleens from different animals throughout the greater part of a year (Fig. 2). Contrary to my expectations I found the weight of the spleen to be by far the greatest in the animals asleep in the pre-hibernating period, while the weight of the spleen in deep-hibernating animals was nearly the same as that found in the 'summer animals'. I take this increase in weight of the spleen as indicating an accumulation of red cells in this organ, and this accumulation as being at its maximum in the sleeping, pre-hibernating animals.

Effects of this accumulation of red cells in the spleen on the hæmatocrit and red cell number were measured, and here again the results were rather conspicuous, as both hæmatocrit and red cell number were decreased for 'October–November animals' as compared with the 'winter animals', being almost the same as those for 'summer animals'. This change in red cell status during the pre-hibernating period is the same whether the animals are awake or asleep. It seems as if the course of events is not exactly the same in this period, comparing the status of red cells in the blood and the accumulation of red cells in the spleen, since in the latter case there is a great difference in the weight of the spleen when the animals are awake and asleep (Fig. 2).

This apparent discrepancy provides the key to further considerations. The possibility exists that a destruction of red cells takes place in the pre-hibernating period, and that the spleen acts as an intermittent reservoir in this period, taking over parts of red cells from the circulation for further transport to the macrophage cells in the liver. This would explain why the weight of the spleen is greatest in the pre-hibernating period and afterwards decreases. Further, it would explain the decrease in the hæmatocrit and the red cell number in both the awake and sleeping 'October–November animals'. Finally, the decreased blood volume observed in awake 'winter animals' would be explained by the destruction of red cells.

Now, the possibility exists that the decrease in hæmatocrit, red cell number, and blood volume could be explained in other ways than by simple destruction of red cells; for example, by accumulation of red cells in some parts of the circulatory system exclusive of the spleen.

The following experimental observations provide an answer to these considerations: A series of blood-volume measurements—combined with parallel hæmatocrit determinations—has been made on animals during induced arousal from the deep-hibernating state. The animals were aroused by taking them from their room (at about 5° C.) to the laboratory (at about 20° C.). Within 12–24 hr. the animals were awake at about 35° C. body temperature, and blood-volume determinations were made according to the Evans blue method mentioned here. The results are given in Fig. 1, where the fully-drawn lines give the blood volumes of sleeping and awake animals, and the stippled line the blood volume during different stages of arousal. In Fig. 1 attention is directed to the following: (1) The blood volume of awake 'winter animals' does not reach the same level as that of the 'summer animals'. About one-third of the blood is lost. (2) There is a rapid increase in the blood volume in the early stages of arousal, reaching its final level at the very beginning of this phase.

From (1) the conclusion may be drawn that the decrease in blood volume in the awake 'winter animals' is due to simple destruction of red cells. The suggestion that red cells might accumulate somewhere in the circulatory system of the awake animals does not seem to be very likely in the case referred to, where heart-rate and blood pressure are of the same order in the awake 'winter animals' as in the 'summer animals'[4]. The destruction theory is the more probable as we have observed a pronounced increase in the bile pigment in serum, urine and fæces in the animals in the pre-hibernating period (and to some extent also in the animals in the beginning of the deep-hibernating period). Further, in some of the 'October–November animals' we found the lowest hæmatocrit values ever observed during the course of the present experiments, and these values reveal a decrease in red cells (other things being equal) of about 30 per cent.

However, the observed loss of blood by destruction of red cells does not tell the whole story about the decrease of blood volume observed in the sleeping 'winter animals' (Fig. 1).

Most likely, the data obtained are a consequence of the method used. At low heart-rates the blood flow is extremely slow and the injection of Evans blue in the circulatory blood, and more particularly the distribution of the dye in the plasma, takes a great deal of time. Now, there is a limit to this distribution, due to the loss of Evans blue from the blood by diffusion. In the experiments referred to in this article the limit was about 3 min. in the deep-hibernating animals. It seems rather likely that a full distribution does not take place all over the circulatory system during this time (and under this condition), and we should therefore like to name the volume calculated from the experiments on deep-hibernating animals 'the Evans blue volume', rather than the exact blood volume. When increase in heart-rate during arousal and as a consequence a more rapid blood flow is obtained, the distribution of the dye should be more quantitative and 'the Evans blue volume' calculated should increase to the value of the true blood volume (Fig. 1, 'arousal').

Now, red cells represent only half the blood. What about the rest of the blood lost, or more particularly, the plasma? Here again the results obtained by hæmatocrit determinations are of importance. In the pre-hibernating period, we were much surprised to find a rather great scattering of the values, with variations from 36 as a minimum to 56 as a maximum. However, as more results were obtained both from animals in this period and the following deep-hibernating period, the scattering in the hæmatocrit (as well as in the red cell number)

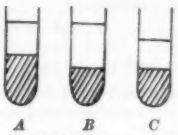

Fig. 3. The relation between plasma and red cells
A, in 'summer animals'; *B*, in 'October–November animals'; *C*, in 'winter animals'

became more easily explicable. The following hypothesis is based on these results as well as those on blood serum and urine obtained by experiments going on in this Laboratory at the time, but not referred to here.

With regard to the blood status during the prehibernating period, two processes are going on with some lag in time. The first one is the destruction of red cells already discussed, and thereupon follows an excretion of plasma substances (water, salts, etc.). In the very beginning of the period only destruction of red cells is going on, and the hæmatocrit decreases (Fig. 3). This should explain the lowest values obtained and referred to here. Afterwards the excretion of plasma begins, and the hæmatocrit increases until the normal values are reached, as in the deep-hibernating animals. The net result is a decrease in total blood volume.

My answer to the question raised at the beginning of this article should be then that the blood volume is decreased mainly by way of red cell destruction and ensuing plasma excretion. The spleen is functioning as an intermittent reservoir regarding the accumulation—and perhaps also the destruction—of red cells. 'The Evans blue volume' reported in this article is about one-third of the total blood volume in 'summer animals', but must be considered as a false datum. More likely this volume reveals pronounced differences in blood-flow in different vascular areas.

At this stage further questions arise, and more detailed determinations are called for, the most prominent ones being: (1) the part played by the kidney in the regulation of blood volume observed; (2) the determination of the life-time of red cells both in 'summer animals' and 'winter animals'; (3) the proportion of new and old red cells in the same animals; (4) investigation on complement components during the whole cycle of hibernation, to look for any connexion between these components and the destruction of red cells observed.

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ROLE OF WATER-REGULATING MECHANISMS IN AMPHIBIAN MORPHOGENESIS: A QUANTITATIVE HYPOTHESIS

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WATER is the only constituent of the amphibian embryo taken up in appreciable quantities from the environment during the course of development, and it accounts for almost three-quarters of the volume of the fully formed embryo. Growth of the embryo and changes in its shape would therefore be expected to involve the uptake or redistribution of water. In the development of *Xenopus laevis*, for example, variations in water content account for almost all the changes in volume that occur, and moreover the water taken up is not uniformly distributed throughout the embryo. It is taken up first into the blastocœl, then into the archenteron and, finally, after the latter has collapsed, into the cells themselves[1]. This can be demonstrated by measurements of the density of the embryo and of its weight in water (reduced weight). The latter remains almost constant until the early tail bud stage, hence all but a small proportion of the volume changes which occur must be the result of water uptake. (Any change in density of the other embryonic constituents would produce a corresponding change in the reduced weight.) A similar conclusion was reached by Rappaport in his work on the growth of explanted amphibian tail buds[2].

Fig. 1 shows the volume of intact *Xenopus* embryos of different ages calculated from the density measured in a density gradient, and the reduced weight, measured on a Cartesian diver balance. It will be noticed that the rate of water uptake increases with age until the eighteenth hour, when there is a sudden decrease in volume resulting from the spontaneous emptying of the archenteron cavity. A similar plot of cell volume (calculated from the reduced weight and the density of opened embryos minus the contents of their intercellular cavities) illustrates the change in distribution of water between the cells and the intercellular cavities (Fig. 1, open circles).

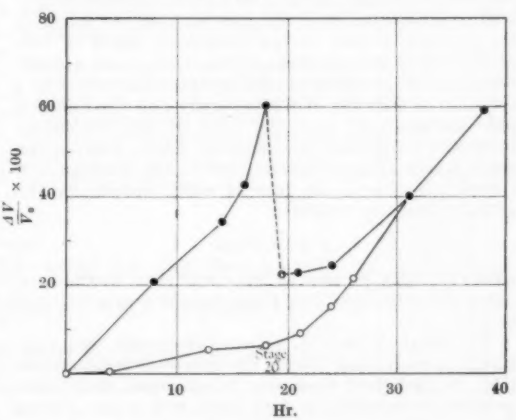

Fig. 1. Changes in volume $\frac{(V_t - V_0)}{V_0}$ of *Xenopus laevis* embryos (open circles) and their cells (closed circles) during development at 25° C. based on reduced weight and density measurements.

$$\text{Volume intact} = \frac{\text{reduced weight}}{\rho_{\substack{\text{intact}\\\text{embryo}}} - \rho_{\text{water}}} \qquad \text{Volume cells} = \frac{\text{reduced weight}}{\rho_{\substack{\text{open}\\\text{embryo}}} - \rho_{\text{water}}}$$

Each point represents the mean of 5 embryos

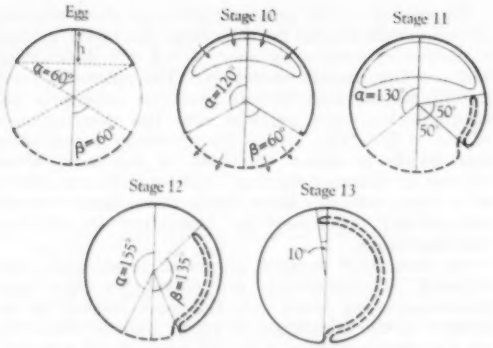

Fig. 2. Diagrammatic summary of morphological data from the *Normal Tables of Xenopus laevis*. Solid line represents the surface of the presumptive ectoderm and neural plate, and the broken line represents the surface of the presumptive endoderm.

Measurement of the freezing-point depression of samples taken from the blastocœl and archenteron of single embryos has shown that the osmotic gradient between the environment and the intercellular cavities decreases with the age of the embryo, and therefore cannot by itself account for the observed water movements[2]. An alternative explanation has been suggested based on the notion that the water-regulating mechanisms of the cells give rise to a net influx of water through the roof of the blastocœl and a net efflux through the cells of the vegetative pole of the embryo[3]. If this notion were correct a difference in the magnitude of the two fluxes would account for the accumulation in the blastocœl; the invagination of the endodermal surface would itself account for the filling of the archenteron, and the increase in area of inwardly polarized ectoderm would account for the observed increase in the rate of water uptake.

This idea has been helpful in interpreting the morphogenetic effects of β-mercaptoethanol on the *Xenopus* embryo[4,5], and the chick blastoderm[6]. I have therefore attempted to present it as a quantitative hypothesis that can be tested experimentally.

If it is assumed that the accumulation of water in the embryo is due to the combined effect of two oppositely polarized areas of the embryonic surface, then the rate of water uptake by the embryo will be a function of the area of the inwardly polarized animal pole surface (A_a) and the area of the outwardly polarized vegetative pole surface (A_v). During the early stages of development, before the blastopore is completely closed, the rate of water uptake by the embryo may be written:

$$f'A_a - f''A_v \qquad (1)$$

where f' and f'' are appropriate rate coefficients. After the closing of the blastopore the rate is equal to $f'A_a$.

The areas A_a and A_v change absolutely and relatively to each other during the course of development (Fig. 2), and their sizes may be estimated from independent morphological data contained in the *Normal Tables of Xenopus laevis*[7] which give values for the radius of the embryo, and the angle subtended by a line joining the boundary of the relevant areas to the centre of the embryo and the animal vegetal axis. It is therefore convenient to express A_a and A_v in terms of these angles:

The surface of a spherical segment $= 2\pi rh$.

For animal pole surface:

$$h = r(1 - \cos\alpha)$$

and $A_a = 2\pi r^2 (1 - \cos\alpha)$

Similarly $A_v = 2\pi r^2 (1 - \cos\beta)$

where α and β are the angles subtended by the boundary of the animal pole surface and the vegetative pole surface respectively.

Equation 1 then becomes:

Rate of water uptake =

$$2\pi r^2[f'(1 - \cos\alpha) - f''(1 - \cos\beta)] \qquad (2)$$

For the purpose of this argument only two parts of the egg surface need to be considered; first, the area from which the ectodermal surface of the neurula is derived—the pigmented area at the animal pole; and secondly, the corresponding area of the vegetative pole surface from which the lining of the archenteron is derived. The area around the equator is excluded from the argument, because it is the zone of transition between areas of opposite polarity and because there is no means of determining its rate coefficients. It is worth noting, however, that the presumptive mesoderm which forms intercellular cavities much later in development is derived from a region just below the equator.

The morphological data from the standard tables are summarized in Fig. 2. If the values of cos α are plotted against time, a straight line is obtained which shows that the area of the animal pole surface increases at a constant rate from cleavage onwards. A similar plot of values for cos β shows, on the other hand, that area of the vegetative pole surface does not change until stage 10, after which it too increases at a constant rate until stage 13, when it extends as a double hemisphere half-way round the embryo and forms the lining of the archenteron. As both these variables change in a linear fashion, intermediate values of cos α and cos β may be obtained by interpolation.

Although the value of the rate coefficients f' and f'' cannot be determined individually from the present data, it is known that, just prior to the collapse of the archenteron, the surface of the ectoderm and surface of the endoderm have approximately the same area, and since the volume of the cells is almost constant at this stage, all the water entering through the ectoderm must be passing through the endoderm. Therefore at this stage $f' = f''$.

If it is now assumed, as a first step, that f' and f'' are constant throughout development then f' and $f'' =$ constant K and equation 2 becomes:

$$\text{Rate of water uptake} = K2\pi r^2(\cos\beta - \cos\alpha) \qquad (3)$$

The value of K may be estimated by dividing the experimentally determined rate of volume change at any stage by the appropriate value of $2\pi r^2 (\cos\beta - \cos\alpha)$:

$$K \frac{\text{Slope of volume change curve}}{2\pi r^2(\cos\beta - \cos\alpha)} \qquad (4)$$

Alternatively it may be estimated from the surface area and the experimentally determined rate of volume change at a stage when the blastopore is closed and all the water entering the embryo accumulates in the archenteron, for example, at stage 20. The values obtained by the two methods are given in Table 1. The experimental values for the rate of water uptake of embryos developing at 25° C. were obtained by combining the results of a number of experiments similar to that illustrated in Fig. 1 and measuring the slope of the curve (Fig. 3) drawn by eye through the mean values of V_t/V_0.

The experimental values so obtained and the corresponding theoretical values calculated from equation (3) are shown in Fig. 4. (The theoretical value for 18 hr. was derived from the surface area of the embryo calculated from the linear dimensions given in the *Normal Tables*, assuming that the embryo at this stage is a prolate spheroid.) The absence of theoretical values for stages 13 and 20 stems from the fact that it is not known exactly when the blastopore becomes watertight. For comparison the corresponding theoretical and experimental values for the rate of water uptake by embryos developing at 20° C. are also shown in Fig. 4.

It will be seen that the experimental values, particularly for embryos developing at 25° C., are close to the theoretical values at all stages. Thus the assumptions on which this analysis is based appear to be consistent with the experimental observations. The hypothesis in its present form, therefore, seems to provide an adequate qualitative and quantitative description of some of the morphogenetic changes that occur during the early development of *Xenopus laevis*. There seems to be no reason why it should not also be helpful in explaining the growth and maintenance of the fluid content of other intercellular cavities, for example, the neural tube, eye cup, cœlom and amniotic cavity, and perhaps in describing the changes in shape of cells and embryonic organs.

I am grateful to Prof. Michael Swann in whose Department this work was carried out, and to my colleagues for their help, in particular Dr. P. D. Mitchell, Dr. H. Kacser and Dr. P. C. Croghan. I am also very grateful to the Nuffield Foundation, which made the project possible.

Table 1

	Stage	Age (hr.)	Experimental rate of water uptake (mm.³/hr.)	Theoretical effective area $2\pi r^2(\cos\beta-\cos\alpha)$ (mm.²)	K (mm./hr.)
		0	—	—	—
		2	0·000	0·65	—
		3	0·018	1·00	0·018
		4	0·028	1·43	0·020
		5	0·034	1·71	0·020
	8	6	0·044	2·02	0·022
		7	0·053	2·55	0·020
		8	0·065	2·98	0·022
	10	9	0·064	3·2	0·020
		10	0·049	2·6	0·019
	11	11	0·033	1·66	0·020
		12	0·026	1·42	0·018
				Mean	0·020
(2)	20	18	0·16	Mean area of Experimental embryos 7·3 mm.²	0·022

Rate of water uptake per unit area. K: (1) calculated from equation (4) using morphological data from the *Normal Tables* (ref. 6), and rates of volume change estimated from the slope of graph in Fig. 3; (2) K calculated from the rate of volume change at 18 hr. and the mean total area of the experimental embryos at this stage.

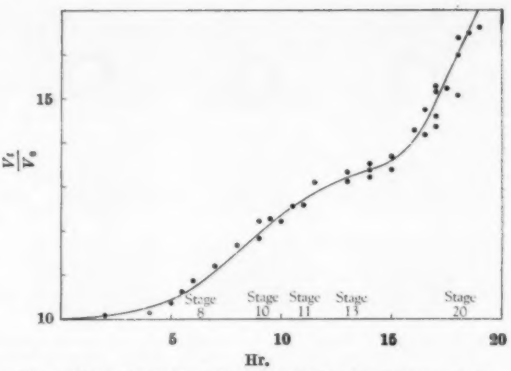

Fig. 3. Changes in volume (V_t/V_o) of intact embryos from a series of experiments at 25° C. used to obtain a measure of the rate of volume change at different stages (see text). Mean volume at T_o = 1·25 mm.³.

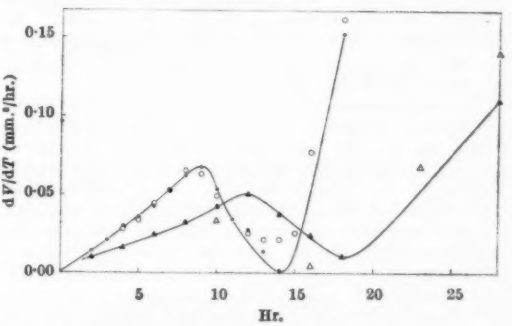

Fig. 4. Theoretical values for the rate of volume change at 25° C. (circles) and 20° C. (triangles) at various stages in development based on the hypothesis described in the text, and the experimentally determined rates derived from the volume changes in Fig. 3 (open circles and open triangles).

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GROWTH HORMONE CONTENT OF SERUM IN INFANTS, CHILDREN, ADULTS AND HYPOPITUITARY DWARFS

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THE preparation of purified human growth hormone (HGH) from human pituitaries (Raben, Li) has made it possible to develop an immunological assay of human growth hormone by means of a human growth hormone rabbit antiserum.

The hæmagglutination-inhibition method is sensitive enough to detect human growth hormone down to 0·2 μgm./100 ml.[1]. The validity of the results depends chiefly on the specificity of the human growth hormone antiserum and the purity of the hormone preparation. That human growth hormone has antigenetical properties has been repeatedly shown by antibody formation in the rabbit[1–10], and by anaphylactic reactions in the guinea pig[4].

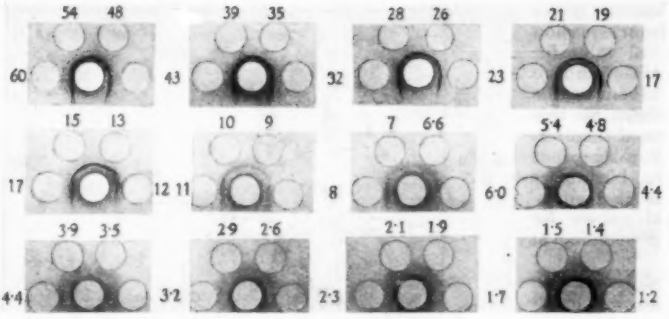

Fig. 1. Precipitin reaction between undiluted human growth hormone/rabbit antiserum (titre 1 : 800) and hormone dilutions (human growth hormone–Raben), as determined by the Ouchterlony technique. Dilutions of growth hormone, in μgm./ml., are arranged clockwise around the central well, which contains the antiserum

When human growth hormone and its specific rabbit antiserum are tested by the gel-diffusion method of Preer[2], the precipitin ring test[7,9], the Ouchterlony technique[7], immunoelectrophoresis[8,9] and by the hæmagglutination-inhibition test[1–3,10], they appear to be homogeneous.

Human growth hormone can be localized in sections of human anterior pituitaries and eosinophilic adenomas in the eosinophilic cells by means of fluorescent rabbit antibodies[6]. This can be regarded as further proof of specificity. Small amounts of contaminating antibodies cannot be entirely excluded by the methods mentioned; however, because the antiserum is diluted for use in the hæmagglutination-inhibition method these possible contaminants can be disregarded. Thyroid stimulating hormone, adrenocorticotrophic hormone and insulin do not interfere with the hæmagglutination-inhibition test of human growth hormone. Tests with the serum of hypophysectomized patients did not show inhibition in the hæmagglutination technique, indicating that only proteins of pituitary origin produce the specific inhibition[3].

Latex agglutination detects growth hormone in amounts down to 0·01 μgm. (ref. 5) and precipitin-ring-test down to 0·1 μgm./0·5 ml. sodium chloride[4]. So far only the hæmagglutination method has been applied to the estimation of concentration of growth hormone in human serum.

The method used for the hæmagglutination inhibition reaction was essentially the one described by Read[1]. Human growth hormone rabbit antiserum was produced by immunization with Raben's hormone preparation[11]. Titres ranged around 1 : 1,000. Sheep erythrocytes were washed in physiological saline, treated with diluted tannic acid and incubated with human growth hormone. With the cells so prepared, the titre of the human growth hormone rabbit antiserum was measured. For use the antiserum is diluted with physiological saline containing guinea pig serum (80 parts : 1 part, v/v). Dilutions of standard hormone solution were added to a constant amount of the diluted antiserum. Simultaneously serial dilutions of the serum to be tested were incubated with the same amounts of the antiserum. If all the antibodies are neutralized by the growth hormone of the serum or the standard solution, hæmagglutination is prevented. With decreasing hormone content of the dilutions, increasing hæmagglutination is observed. The end-point is that concentration of growth hormone at which complete hæmagglutination first occurs. Controls using washed and tanned erythrocytes without human growth hormone coating and hormone-coated cells without antiserum were always negative. The sensitivity of the method can be regulated by varying the antiserum dilution. If the dilution is not carried too far, that is, not quite to the titre-dilution, the end-point of the hæmagglutination-inhibition can be determined exactly. All samples were tested in quadruplicate and gave identical results, even in different assays.

Our own work using the Ouchterlony agar diffusion technique showed one single precipitation line between the undiluted human growth hormone rabbit antiserum and hormone concentrations ranging from 6,000 μgm./100 ml. down to 300 μgm./100 ml. (Fig. 1). No precipitation was seen when human growth hormone was tested against anti-human serum. In contrast a specific antiserum against human albumin with a high titre in one test produced a precipitation line, but only if growth hormone was used in a concentration of 100 mgm./100 ml., an amount far greater than that used in the hæmagglutination technique. On subsequent trials this observation could not be reproduced. This finding could be interpreted as indicating contamination of the growth hormone preparation with small amounts of albumin.

The growth hormone concentration in the serum of normal children and adults in our series using the hæmagglutination-inhibition technique varied between 24 μgm./100 ml. and 60 μgm./100 ml. for children and between 16 μgm./100 ml. and 36 μgm./100 ml. for adults (that is, 240 mμgm./ml.–600 mμgm./ml. and 160 mμgm./ml. and 360 mμgm./ml.). The mean values for children (42 children without cord-blood values: 39·6 μgm./100 ml., standard deviation 10·48) are significantly greater than the mean concentration in adults (18 adults: 26·0 μgm./100 ml., $S.E. = 5·53$), $P = 0·001$. The mean value of growth hormone in puberty (14 children from 11 to 17 years) is 43·43 μgm./100 ml. ($S.E. = 8·61$). This value does not differ significantly from the mean growth hormone concentration of 28 children under 11 years of age (without cord-blood values), which amounts to 37·75 μgm./100 ml. ($S.E. = 10·94$) with $P = 0·1–0·05$.

In nine specimens of cord-blood the values ranged from 24 to 36 μgm./100 ml. (that is, 240–360 mμgm./ml.).

Fig. 2 shows the results in relation to the age of the individuals tested. There was no clear relation between the growth hormone concentration at a certain age and the rate of gain in height of the corresponding period. There is a slight increase in the mean growth hormone concentration between the age of 11 and 17 years (puberty), which is not significant. In contrast, values of growth hormone in 18 adults showed an average which is significantly lower than the average during childhood.

In three hypopituitary dwarfs we found concentrations of growth hormone which are well below the lowest values encountered in normal children and adults. In this group a 6-year-old boy and a 13·5-year-old girl had a concentration of 6 μgm./100 ml., a 14·5-year-old male dwarf, who previously had

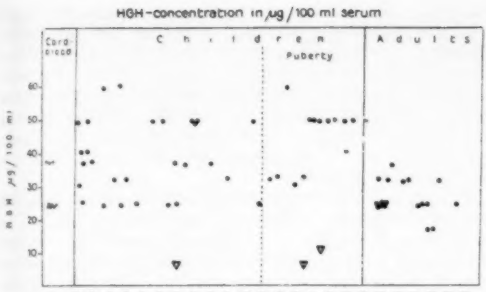

Fig. 2. ●, normal individuals; △, hypopituitary dwarfs

been treated with injections of growth hormone, showed a concentration of 10 µgm./100 ml. after treatment had been discontinued for 10 days.

Our findings for the growth hormone content in normal human serum correspond with the values given by Read (16–64 µgm./100 ml.). The content in the samples of cord-blood also falls in the range reported by him (24–48 µgm./100 ml.). In our three hypopituitary dwarfs the concentrations of growth hormone were a little higher than Read found in a similar group, but they still are clearly below the normal range.

Whereas Read believes that there is no difference in the content of serum between children and adults, our mean values for children are significantly greater than the mean concentration in adults. Investigations are under way to settle the question of the suggestive increase in the concentration of human growth hormone during puberty.

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SPERMATOCYTOGENESIS WITH POSSIBLE SPERMIOGENESIS OF GUINEA PIG TESTICULAR CELLS GROWN *IN VITRO*

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SPERMATOGENESIS has been described as having two phases. In the first phase, spermatocytogenesis, the germ cells undergo repeated mitoses and meiosis followed by structural changes which give rise to a new type of cell, the spermatid. In the second phase, spermiogenesis, the spermatids undergo a series of complex transformations which result in the formation of mature spermatozoa.

In general the three cell types of the testes considered of major importance in spermatogenesis are the spermatogenic or germ cells, the Sertoli or sustentacular cells, and the interstitial or endocrine cells. Recently, we have been able to propagate testicular cells continuously in culture and have identified these three cell types by morphological and histochemical methods. Moreover, it appears that spermatogenic cells in culture retain the maturation potential needed to form spermatids and possibly spermatozoa *in vitro*.

In experiments reported elsewhere[1] it was shown that testicular cells continuously propagated in cell culture contain antigen(s) required to induce experimental allergic aspermatogenesis in the guinea pig and that testicular cells carried through several fluid transfers continued to synthesize antigen(s) which react (Schultz-Dale) with sensitized ilea of guinea pigs in which allergic aspermatogenesis has been induced.

The purpose of this article is to describe the cytological characteristics of testicular cells grown in cell culture.

Testes were aseptically removed from mature albino guinea pigs, washed in warm (37° C.) *tris*-buffered saline and stripped of the tunica vaginalis. The testes were minced with scalpel blades and treated with 0·25 per cent trypsin in *tris*-buffered saline at 37° C. for 2–3 min. After trypsinization the cells were diluted with 10 vol. of cold *tris*-buffered saline to stop the action of trypsin, centrifuged at 2,500 r.p.m. for 3 min. and re-suspended in nutrient medium to a final concentration of approximately 2×10^6 cells/ml. 10 ml. of the cell suspension were used to seed each culture.

The culture medium contained Earle's saline, 'Millipore'-filtered horse serum, lactalbumin hydrolysate, chick embryo extract, a complex vitamin mixture and antimicrobial agents. Glutamine was added to the cultures immediately before cultivation.

Cells were transferred by treating the parent culture at 37° C. for 3–5 min. with 0·25 per cent trypsin in *tris*-buffered saline. After trypsinization cells were washed once with cold *tris*-buffered saline and re-suspended in warm medium so that each screw-capped milk dilution bottle received 10 ml. containing approximately $2{\cdot}0 \times 10^5$ cells. Cells were grown in a humidified incubator at 37° C. in an atmosphere of 10 per cent carbon dioxide and 90 per cent oxygen.

Single cells for photomicrographic and histochemical analysis were obtained by diluting cell suspensions to approximately 5 cells/ml. 1 ml. was placed in a Petri dish containing several coverslips. After

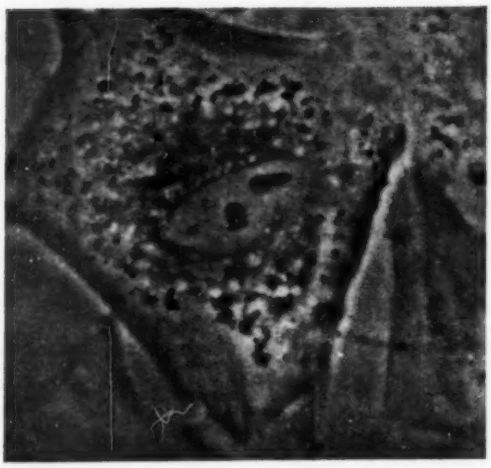

Fig. 1. Sertoli cell in culture 37 days. Phase contrast (× 1,188)

Fig. 2. Interstitial cell after 3 *in vitro* transfers: in culture 18 days. Phase contrast (× 250)

attachment to coverslips, cells were examined by phase microscopy or stained and photographed. Morphological and histochemical criteria used in identification of the different cell types were those already described and confirmed by others for the intact testis[3–6]. Coverslip preparations were fixed in 10 per cent neutral formalin for 8–10 hr. A triple stain for deoxyribonucleic acid, polysaccharides and proteins was used for differentiating between spermatogenic cells and other cellular components of the testes[7]. The nuclei stained blue to green, the mucus (polysaccharides) and structures in the Golgi zone, red; and the cytoplasm yellow. Thus the 'idiosomic' granules of the Golgi region in the primary and secondary spermatocytes, which later fused to form the 'acrosome' and 'head cap' of the spermatids, could be followed through various stages to the mature sperm by the bright red staining properties of the polysaccharide[2,5,6].

Fig. 1 is a coverslip preparation containing an epithelial-like cell with an ovoid nucleus and three nucleoli. The nuclear membrane is clearly outlined and the reticular cytoplasm contains fibrillar mitochondria, chromatin granules and lipoid droplets. This cell type is characteristic of the Sertoli cell and retains its morphological and histochemical properties during repeated passage in culture. Multiplication of the Sertoli cell *in vitro* results in a 'Sertolian syncytium' or a confluent growth connected by protoplasmic bridges.

Fig. 2 is a coverslip preparation of an epithelioid cell which contains a slightly elongated nucleus. The cytoplasm is highly granular and contains various inclusions. The perinuclear cytoplasm contains highly refractive, discrete, round globules, fairly uniform in size, which react positively when tested for neutral fats and lipoids. This cell type characteristic of the interstitial cell has been described in detail by Rasmussen[2].

The large spherical or oval cell type seen in Fig. 3 grew as a free-form, unattached, monodispersed cell suspended in the growth medium and contained a spherical mass close to the nucleus which stained red and is characteristic of primary and secondary spermatocytes. Primary spermatocytes (Fig. 3, *A*) are the largest of the developing germ cells. Secondary spermatocytes (Fig. 3, *B*, *C*), derived from the primary spermatocytes by the first reduction division, are somewhat smaller than the primary and generally the chromatin is less dense. Cells *B* and *C* have resulted from the division of what appears to have been a primary spermatocyte similar to *A*. Each of these secondary spermatocytes presumably will undergo complex structural changes to form spermatids.

Fig. 4 contains a group of cells, one of which (*A*) appears to be in early metaphase with tetrads typical of meiosis. The structures in this cell suggest the occurrence of parasynapsis with the organization of paired homologous chromosomes and the formation of quadripartite tetrads of chromosomal configurations. The presence of this cell distinctly separates meiosis *in vitro* from mitosis. *B* appears to be a spermatid flattening at the beginning of the maturation phase with the acrosomic granule transformed into a cone. The tadpole-like structure

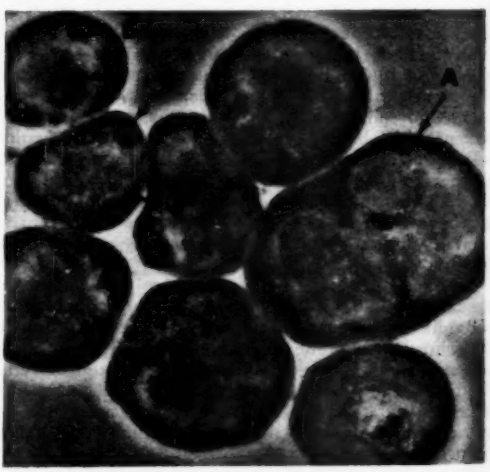

Fig. 3. Primary and secondary spermatocytes growing in supernatant fluid after 3 *in vitro* transfers: in culture 35 days. Phase contrast (× 1,188)

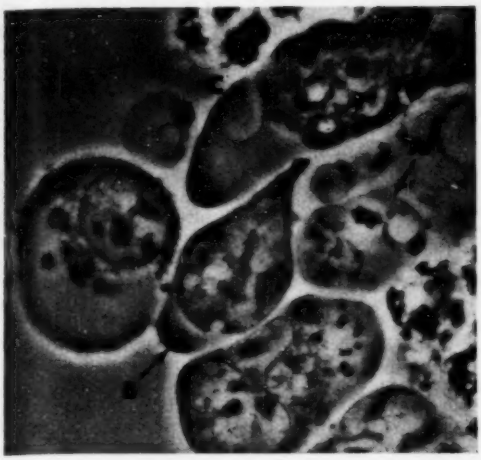

Fig. 4. A cell in meiosis (*A*) and spermatids in different stages of formation after 3 *in vitro* transfers: in culture 35 days. Phase contrast (× 1,188)

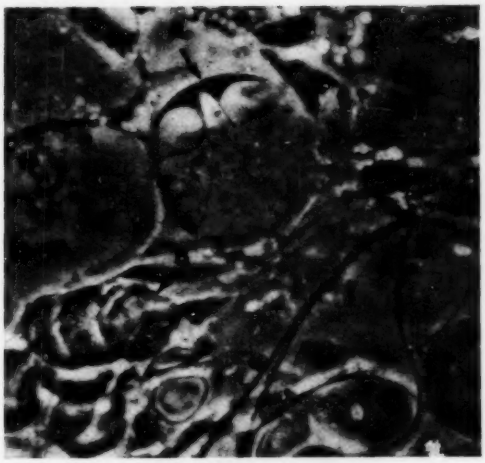

Fig. 5. Structures which appear to be mature and immature spermatozoa. Phase contrast (× 1,188)

(Fig. 4, *C*) is a spermatid with a profile view of the head cap.

In Fig. 5, *A* is a round cell which appears to contain the hollow heads of two sperms, while at the lower left portion of Fig. 5 are structures which appear to be mature sperm.

These findings show that spermatogenic, sustentacular and interstitial cells of the testes continuously propagated in culture retain their morphological and histochemical characteristics in addition to their immunological properties[1]. Moreover, it appears that spermatogenic cells carried in culture undergo metamorphosis to form mature sperm. It is important to know in such work as this whether spermatogenic cells taken from the testes were in an advanced stage of development and merely 'matured' into spermatozoa in culture or whether immature spermatogonia underwent all the necessary transformations to form mature sperm. This problem is now being investigated by time-lapse cinematography.

This work was supported by funds from grant *CY*–5188, National Institutes of Health and the Population Council, New York.

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FORMATION OF DIFFERENT HÆMOGLOBINS IN TISSUE CULTURE OF HUMAN BONE MARROW TREATED WITH HUMAN DEOXYRIBONUCLEIC ACID

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ALTHOUGH transformation of bacteria is an established fact, the transformation of mammalian cells is in a pioneering phase of research. In order to accomplish transformation, isolated deoxyribonucleic acid (DNA) must cross the cell membrane and become part of the genetical apparatus of the cell, thereby regulating the production of a substance within that cell which was absent before entry of DNA. It has been demonstrated[1] that intact DNA enters the mammalian cells and does become part of the nuclear material. The presence of a substance in the cell synthesized as a direct result of the new DNA is more difficult to demonstrate. In the human hæmoglobins each polypeptide chain synthesized is directly related to the presence of a gene or sequence of DNA in the chromosomes of the erythrocyte precursors. This was demonstrated by Itano and Robinson[2]. Incorporation of DNA from cells producing one type of hæmoglobin into cells which produce another kind of hæmoglobin should allow the detection of a new hæmoglobin in the cells into which the DNA has been incorporated. Production of hæmoglobins not previously present in tissue culture following incorporation of DNA into the erythrocytic series of cells has been accomplished here. These new hæmoglobins contain the polypeptide chains α^A, β^A, β^S, γ and probably an altered α-chain.

Bone containing red marrow obtained at surgery from an individual, homozygous for hæmoglobin *A*,

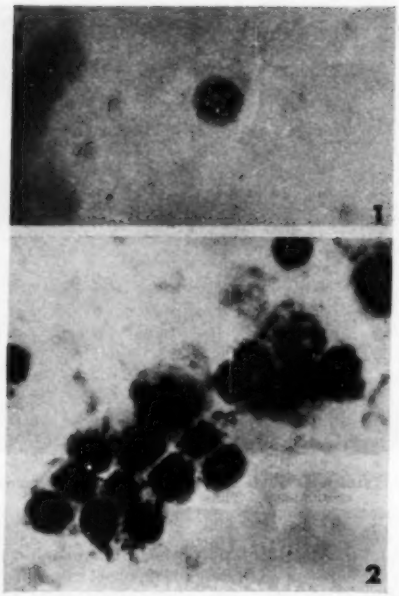

Fig. 1. Autoradiogram using Kodak '*AR* 10' stripping film. Exposure, 41 days. Stained with Wright-Giemsa. 1, mitotic figure showing incorporation of tritiated thymidine into the chromosomes of cells from bone from an individual with hæmoglobin *A* after 4 days of tissue culture. 2, group of prorubricytes and rubricytes showing incorporation of tritiated DNA into cytoplasm and nucleus of bone marrow from a sickle-cell anæmia patient after incubation for 24 hr. with tritiated DNA

was incubated in Tyrode solution containing 10 per cent of the patient's plasma. Methyl-labelled tritiated thymidine, 500 μc. in 0·02 mgm./0·5 ml. (New England Nuclear Corp.) was added to the tissue culture. After maintaining the tissue culture at 27° C. for four days, autoradiograms of the marrow cells were prepared using Kodak '*AR* 10' stripping film (Fig. 1, 1). DNA was extracted by the salt extraction method used by Spizizen[3]. The extracted DNA gave typical absorption spectra[4] and the concentration calculated by $E_{1\,cm.}^{1\%} = 192$ at 260 millimicra was 1 μgm./μl. Nine hundred μgm. of DNA were added to bone marrow obtained by sternal puncture from a patient with sickle-cell anæmia. The hæmoglobin present was homozygous hæmoglobin *S* with 8 per cent fœtal hæmoglobin. This bone marrow and the added DNA were maintained in tissue culture for 10 days at 27° C. in a sealed culture dish in Tyrode solution containing 10 per cent of the patient's plasma. A plasma clot formed during the course of the incubation. Cells were removed at 16, 24 and 48 hr. after the addition of DNA and slides were prepared with Kodak '*AR* 10' stripping film for autoradiographical examination. All autoradiograms showed incorporation of the tritium-labelled DNA into the cytoplasm or the nucleus of nucleated erythrocytes (Fig. 1, 2), and into some other marrow cells. After 10 days, an aliquot of patient's plasma labelled with iron-59 as ferrous chloride was added and the tissue culture was incubated under continuous shaking in a water-bath at 37° C. for 1·5 hr. The cells were washed with Tyrode solution and a hæmolysate prepared which was fractionated by electrophoresis in *p*H 8·6 veronal buffer. The total hæmoglobin component was found to be labelled with iron-59 and contained 45 per cent of the total radioactivity on the paper strip. The specific activity was 100 counts/min./mgm. of hæmoglobin. These results agree well with previous experiments reported in ref. 5. This demonstrates that at the end of 10 days of tissue culture the erythrocyte cells were capable of synthesizing labelled hæmoglobin.

Electrophoresis was used to compare the cell hæmolysate from the tissue culture of DNA-treated cells with the cell hæmolysates from peripheral blood of the individual homozygous for hæmoglobin *A* (source of the DNA) and the patient with homozygous hæmoglobin *S* with 8 per cent hæmoglobin *F* (the original tissue culture). The results are in Table 1.

Fig. 2 depicts the results of agar electrophoresis showing the different hæmoglobins formed in tissue culture treated with DNA.

Electrophoresis in 7 *M* urea–0·1 *M* veronal buffer at *p*H 8·2 separates the polypeptide chains of hæmoglobin[5]. This method was used to separate the polypeptide chains of the new hæmoglobins, hæmoglobin *A* and hæmoglobin *S*–*F* found in the cell hæmolysates (Fig. 3). The identification of the

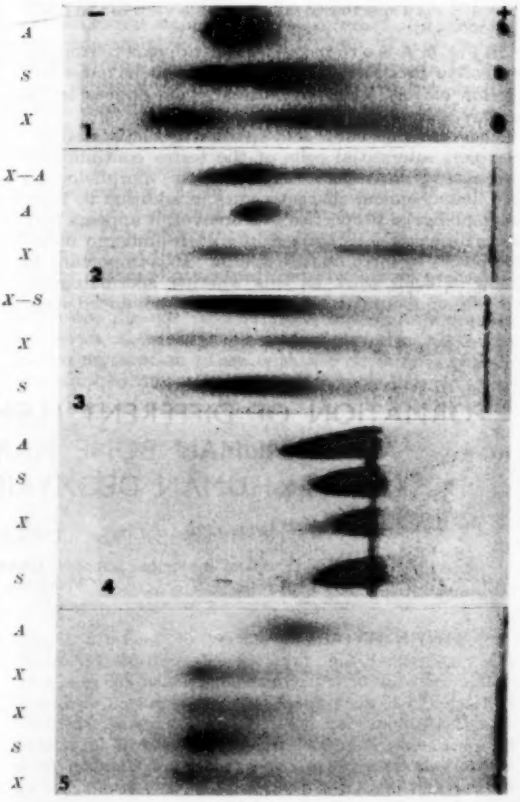

Fig. 2. Agar electrophoresis of cell hæmolysates from tissue culture treated with DNA, labelled *X*, from peripheral blood of the sickle-cell anæmia patient, labelled *S* (original bone marrow tissue culture) and of an individual with hæmoglobin *A*, labelled *A* (source of DNA). All material was in the form of cyanmethæmoglobin and was of equal concentration. 1, 2, 3, *tris*-EDTA-boric acid *p*H 7·1; 4, *tris*-citric acid *p*H 6·1; 5, *tris*-EDTA-boric acid *p*H 8·6. The origin is designated by a pencil mark. Stained with bromphenol blue

polypeptide chains present in the cell hæmolysates was accomplished by electrophoretic comparison with purified α^A, β^A, β^S and β^C polypeptide chains isolated by countercurrent distribution[9].

The absorption spectra in the range 200–700 mμ for all the above cell hæmolysates were superimposable and typical for oxyhæmoglobin containing a small amount of methæmoglobin.

From the results of these experiments it appears that cells which were genetically determined to synthesize only α^A, β^S and γ polypeptide chains of hæmoglobin, on incorporation of the genetical material (DNA) from cells which synthesized only α^A, β^A and γ polypeptide chains, now are able to synthesize the originally present α^A, β^S and γ polypeptide chains and in addition β^A and possibly an altered α polypeptide chain. These polypeptide chains in the cells altered by DNA combine to form two hæmoglobins differing electrophoretically from hæmoglobins *A*, *S* and *F*.

Table 1. ELECTROPHORESIS OF THE HÆMOLYSATES (HÆMOGLOBIN *X*) OF THE TISSUE CULTURE HUMAN BONE MARROW TREATED WITH DNA COMPARED WITH CONTROLS OF HÆMOGLOBIN *A* (SOURCE OF THE DNA) AND HÆMOGLOBIN *S*–*F* PER CENT (BONE MARROW BEFORE TREATMENT WITH DNA)

Whatman 3 *MM* Filter Paper	−	Origin*	+
Veronal			*A*
*p*H 8·6, 0·05 *M*			*X*
			S
Tris-citric acid	*A*		
*p*H 6·1 (ref. 6)	*X*		
	S		
	X		
Phosphate	*A*		
*p*H 6·8, 0·05 *M*	*X*		
	S		
	X		
Phosphate	*A*		
*p*H 6·5, 0·05 *M*	*X*		
	S		
	X		
Tris-ethylenediamine tetraacetic acid (EDTA)-boric acid			*A*
*p*H 8·9 (ref. 7)		*X*	
		S	
		X	
Agar (ref. 6)			
Tris-EDTA-boric acid	*A*		
*p*H 8·6	*X*		
	S		
	X		
Tris-EDTA-boric acid	*A*		
*p*H 7·1	*X* *X*		
	S		
	X *X*		
Tris-citric acid	*A*		
*p*H 6·1	*X*		
	S		
	X		

All cell hæmolysates are of equal concentration and in the form of cyanmethæmoglobin.

* Origin represents the point of application of the samples, + and − the electrodes and the positions *X*, *S*, *A* designate the relative mobilities of the hæmoglobins found in the previously described cell hæmolysates.

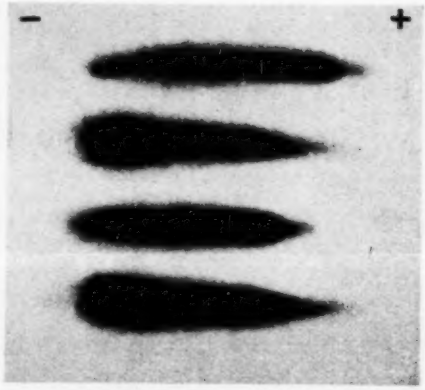

Fig. 3. Electrophoresis on Whatman 3 *MM* paper using 7 *M* urea-0·1 *M* veronal *p*H 8·2, 350 V., 5 m.amp. for 5 hr. Hæmolysates as designated in Fig. 2. The electrophoretic mobility of the polypeptide chains of hæmoglobins, in this method, is towards the anode $\beta^A > \gamma > \beta^S$. The α-chains migrate towards the cathode. Stained with bromphenol blue

Bone for the extraction of DNA was made available by Prof. H. Boyd, of the Department of Orthopedic Surgery, University of Tennessee, and Campbell Clinic, Memphis. Bone marrow was made available by Prof. A. P. Kraus, Department of Medical Laboratories, University of Tennessee College of Medicine. I thank them.

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HÆMOGLOBIN α^A

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NORMAL adult hæmoglobin (Hb-*A*) consists of two α^A-polypeptide chains joined to two β^A-polypeptide chains and has a molecular formula $\alpha_2^A\beta_2^A$ (ref. 1). Most human hæmoglobin variants, as well as fœtal hæmoglobin, $\alpha_2^A\gamma_2^F$ (refs. 2, 3), and hæmoglobin A_2, $\alpha_2^A\delta_2^{A_2}$ (refs. 4, 5), have the same type of structure. Two hæmoglobin variants have been described which do not contain α-chains, namely, hæmoglobin *H*, β_4^A (refs. 6–8) and hæmoglobin

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XUM

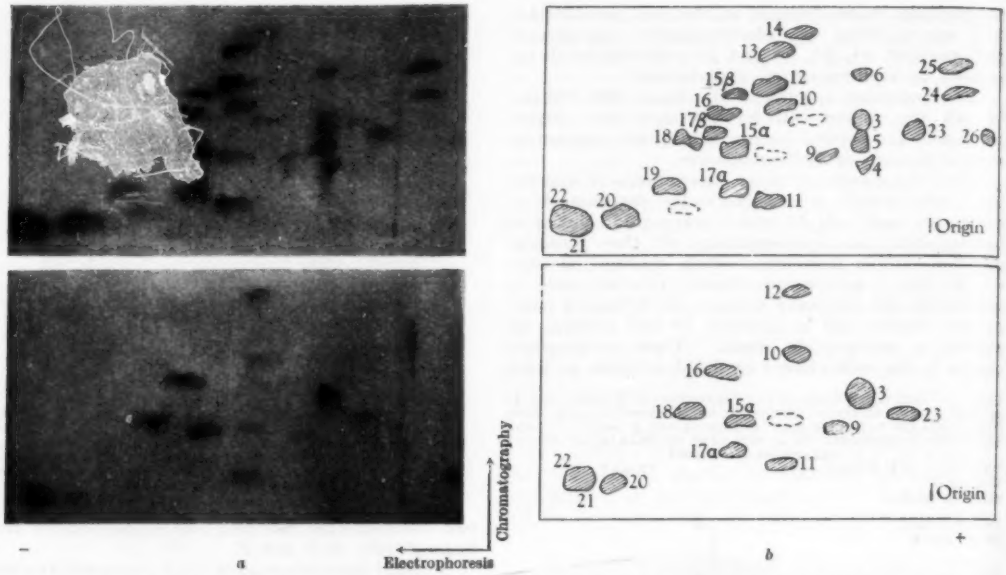

Fig. 1. Analysis of peptides obtained by tryptic hydrolysis of (top) Hb-*A* and (bottom) Hb-α^A; electrophoresis in pyridine, acetic acid, water (90 : 0·4 : 10), *p*H 6·4, followed by ascending chromatography in isoamyl alcohol, pyridine, water (35 : 35 : 27). (*a*) Photographs of peptide pattern stained with ninhydrin; (*b*) tracings of peptide pattern (Method and numbering system as ref. 28)

'Bart's', γ_4^F (refs. 9–11). In this report, the properties of a hæmoglobin, now called hæmoglobin α^A(Hb-α^A), consisting of a single α^A-chain, are described.

Hb-α^A was prepared by eluting purified Hb-*A* from a column of carboxymethyl cellulose using an 0·3 *M* sodium acetate buffer of *p*H 4·7, dialysing the eluted hæmoglobin solution back to a neutral *p*H and then separating the resulting hæmoglobin mixture by starch block electrophoresis[12,13]. In phosphate buffer of *p*H 7·0 and [Na^+] 0·08 *M* Hb-α^A migrated more rapidly towards the cathode than Hb-*A*.

Fig. 1 compares the peptides obtained from the tryptic digestion of Hb-α^A with those obtained from Hb-*A*. It can be seen that those peptides, namely, 4, 5, 6, 12, 14, 15β, 17β, 19, 24, 25 and 26, arising solely from the β^A-chain of Hb-*A* are absent. In contrast, peptides 3, 9, 10, 11, 13, 15α, 17α, 18 and 23 which originate from the α^A-chain appear in the 'fingerprint'. Hb-α^A was also dissociated and recombined[14,15] with canine hæmoglobin, hæmoglobin *G*/*C* and hæmoglobin *I*. In the recombination experiment with canine hæmoglobin, only the slower of the two new species which form in the recombination of canine hæmoglobin with Hb-*A* was observed (Fig. 2). This species is known to have the composition $\alpha_2^A\beta_2^{Can.}$ (ref. 16). When Hb-α^A was recombined with hæmoglobin *G*/*C*, $\alpha_2^G\beta_2^C$ (ref. 17), the only new species formed was hæmoglobin *C*. On the other hand, it is known that when hæmoglobin *G*/*C* is recombined with Hb-*A*, both hæmoglobins *G* and *C* form[17] according to the equation:

$$\underset{\text{Hb-}G/C}{\alpha_2^G\beta_2^C} + \underset{\text{Hb-}A}{\alpha_2^A\beta_2^A} \rightarrow \underset{\text{Hb-}G/C}{\alpha_2^G\beta_2^C} + \underset{\text{Hb-}C}{\alpha^A\beta_2^C} + \underset{\text{Hb-}G}{\alpha_2^G\beta_2^A} + \underset{\text{Hb-}A}{\alpha_2^A\beta_2^A}$$

Thus, the appearance of hæmoglobin *C* but not hæmoglobin *G* in this experiment as well as the formation of the species $\alpha^A\beta^{Can.}_2$ but not the species $\alpha^{Can}_2\beta^A_2$ in the recombination with canine hæmoglobin suggest that the new hæmoglobin consists solely of α^A-chains. Recombination of Hb-α^A with hæmoglobin *I* produced only Hb-*A*, confirming the presence of α^A-chains in Hb-α^A. This experiment is analogous to the recombination of hæmoglobin *H* with a hæmoglobin possessing abnormal β-chains[18,19]. In the latter, hæmoglobin *H* donates β^A-chains which combine with the α^A-chains of a β-chain variant to form Hb-*A*, while in the experiment reported here Hb-α^A donates α^A-chains for the formation of Hb-*A*, this time with the β^A-chains of an α-chain variant.

The sedimentation properties of Hb-α^A (ref. 20) resemble closely those of sperm whale myoglobin and the hæmoglobin of the brook lamprey, *Lampreta fluviatilis*. Thus, the value of 1·7 *S* for the sedimentation coefficient of Hb-α^A may be compared with the figure of 1·7 *S* for myoglobin determined under the same experimental conditions, or of 1·8 *S* quoted for lamprey hæmoglobin[21]. Moreover, the molecular weight of Hb-α^A, 17,000 ± 1,000, is similar to the value of about 18,000 for myoglobin and 17,200 for lamprey hæmoglobin[21] and is near the molecular

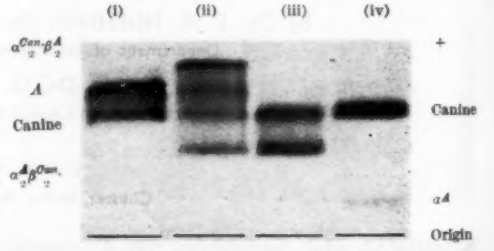

Fig. 2. Analysis in starch gel, *tris* citrate–borate discontinuous buffer system, migration time 2 hr., *o*-dianisidine stain; (i) mixture of Hb-*A* and Hb-canine; (ii) mixture of Hb-*A* and Hb-canine dissociated and recombined; (iii) mixture of Hb-α^A and Hb-canine dissociated and recombined; (iv) mixture of Hb-α^A and Hb-canine

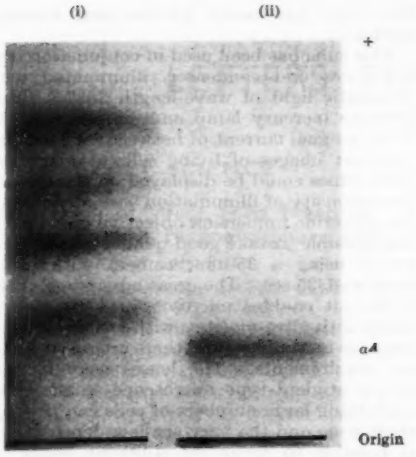

Fig. 3. Analysis in starch gel, *tris* citrate–borate buffer system, migration time 2 hr., *o*-dianisidine stain; (i) hæmolysate containing Hb-*A* + *G* + *C* + *G/C*; (ii) Hb-α^A

weight expected for the α^A-chain on the basis of its amino-acid composition[22,23]. Hb-α^A therefore consists of a single α^A-polypeptide chain.

The absorption characteristics of the cyanmet-derivative of Hb-α^A are, with one exception, identical to those of Hb-*A*. The one difference is found in the wave-lengths of the maximum of the 'protein' band which are at 274 mμ for Hb-*A* and at 270 mμ for Hb-α^A. This shift to lower wave-lengths would, however, be expected for a hæmoglobin consisting only of α^A-chains since the latter have a significantly higher tyrosine : tryptophan ratio than the globin of Hb-*A* itself.

Hb-α^A has a distinctive electrophoretic mobility, migrating nearer to the cathode than hæmoglobin *G/C* at *p*H's between 7·0 and 8·7 (Fig. 3). It is stable in solution, either as the carbonmonoxy or cyanmet-derivative, for a week at 4° C., but gradually precipitates on longer storage. When deep-frozen at − 15° C. it precipitates almost completely from solution in 48 hr.

These experiments have, therefore, shown that a hæmoglobin consisting of a single α^A-chain is capable of separate *in vitro* existence. Moreover, its electrophoretic mobility and stability in solution are such that if it occurred *in vivo*, even in small amounts, it would be detected by starch-gel electrophoresis either in the *tris* citrate–borate system at *p*H 8·7 (ref. 24) or in phosphate buffer[15,25,26]. Starch-gel analyses of fresh hæmolysates from a number of individuals, normal or with hæmoglobinopathies or thalassæmia, carried out by us have failed to reveal any such component. However, it is probable that small amounts of this hæmoglobin are formed in some recombination experiments, particularly if prolonged acid treatment is used. The only other hæmoglobin with an electrophoretic mobility slower than hæmoglobin *G/C* is hæmoglobin Gower 2, found in the red cells obtained from a small human fœtus[27]. However, the position of the tryptophan fine structure band of the latter hæmoglobin is at 2902 Å. which is significantly shorter than that found for Hb-α^A and these two hæmoglobins are therefore not the same. It would thus appear that Hb-α^A does not occur *in vivo* and this is of some importance in any consideration of the synthesis of the hæmoglobin molecule.

This investigation has been supported by a grant from the Colonial Medical Research Committee and also by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council.

We are indebted to Miss Jean Atwater for a sample of a hæmolysate containing hæmoglobin *I* and to Dr. A. Edmundson for a specimen of crystalline sperm whale myoglobin.

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TELEVISION MICROSCOPY

A Flying-spot Interference Television Microscope

AN all-electronic flying spot interference television microscope for the rapid determination of cellular dry mass has been developed in this laboratory. The work has stemmed from the suggestions of Barer[1] and from consultation with F. F. Roberts and F. Smith of C. Baker Instruments, Ltd.

The interferometric technique of dry-mass determination has been well documented[2] and mechanical systems of scanning have been developed[3].

The equipment developed in this laboratory uses the flying-spot technique of scanning. A low-speed

system with 10-sec. sweep is used in order to improve the ratio of signal to noise and to ease the requirements on the computing circuitry. The system consists of the flying-spot scanner tube, interference microscope, multiplier phototube, and a photoformer for the continuous solution of the intensity-phase angle equation. A monitor presents a picture of the photoformer output, which represents the relative phase angle present in the cell and, therefore, the dry mass of the various portions of the cell on a grey scale. The photoformer output is fed to an integrator circuit which sums the phase angle in the entire field. The integration output at the end of each sweep, therefore, represents the total dry mass present in the optical field. The information from the integrator is continuously read out on a strip chart recorder.

Auxiliary pulse generating equipment is so arranged that the integrator may be made to sum only the information in a selected area of the field. The area being integrated is indicated on the monitor by brightening. This area may be a square or rectangle with a minimum size of about 2μ at the magnification at present used.

The grey scale dry-mass images of the living cell may be continuously recorded on 16-mm. motion picture film. When such a film is presented at a speed of 16 frames per sec. a time-lapse motion picture is obtained.

This system has several advantages over previously described techniques. (1) The data on the dry mass of whole cells or segments of cells are obtained and recorded by two methods every 10 sec. (2) The dry-mass images of the specimen are continuously displayed on the monitor tube during the course of the experiments. (3) The visual presentation of the dry-mass image makes the positioning of the brightened area of the raster quite easy and this permits continuous determination of the dry mass of selected areas of the cell.

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Ultra-Violet Television Microscopy

Several attempts to use ultra-violet-sensitive television cameras in association with ultra-violet microscopes to give an immediate display of the image have been reported[1]. From published photographs the sensitivity and image quality do not appear to be very high and results obtained with other types of image converters have also been disappointing. A newly developed E.M.I. 'Vidicon' camera tube incorporating a quartz window and a specially modified arsenic triselenide target layer appears to be very promising. The spectral response curve has a peak at 4000 Å. with a half-intensity band-width of about 1500 Å. The sensitivity at 2537 Å. is approximately 0·10 μamp./μW./cm.^{2}. This appears to be several times higher than previously reported values. The layer has unity gamma and reasonably low inertia.

This tube has been used in conjunction with quartz objectives and condenser, illuminated with monochromatic light of wave-length 2537 Å. from a low-pressure mercury lamp and simple monochromator. With a signal current of between 0·05 and 0·1 μamp., excellent images of living cells at magnifications of 3,000 times could be displayed on a television screen. The intensity of illumination was adequate even with the glycerine immersion objective of N.A. 1·25 and it was possible to take good-quality photographs of the screen using a 35-mm. camera with exposures as short as 1/25 sec. The great advantage of the system is that it enables microscopy to be carried out as easily with ultra-violet as with visible light. Focusing presents no problem and there are no special mechanical requirements. The lenses were mounted on a simple student-type microscope stand. Many fields containing large numbers of cells can be scanned in a short time and the very shallow depth of field makes it possible to focus the different levels through a relatively thick specimen, thus helping to build up a three-dimensional impression.

In this connexion the technique of immersion refractometry[2] has been adapted for ultra-violet work. The lens-like behaviour of an uncompressed spherical cell makes observation of internal structures difficult. This can be partly overcome by immersing such cells in a suitable protein medium having a refractive index approaching that of the cytoplasm. This method has enabled the structure of chromosomes to be seen very clearly in undamaged cells. Since, however, the cytoplasm of most cells shows some degree of absorption at 2537 Å. it is difficult to bring about actual reversal of contrast by immersion in protein alone. If a little nucleic acid is added to a less-concentrated protein solution it becomes possible to match the cytoplasm exactly against the surrounding medium or to make it appear bright instead of dark. This method opens up the possibility of measuring both the refractive index and absorption of living cells in the ultra-violet to obtain quantitative cytochemical information. It also makes it possible to vary at will the contrast of internal detail, a technique referred to elsewhere as "optical dissection"[3]. Finally, the method offers considerable promise for rapid microspectrophotometry. It is quite simple to scan any line across the image of a cell and display a light intensity curve on an oscilloscope screen. There is no difficulty in principle in converting such curves to an optical density scale and in feeding the results to an electronic integrator to measure the total mass of absorbing material.

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LETTERS TO THE EDITORS

SPACE SCIENCE

Interaction of West Ford Needles with the Earth's Magnetosphere and their Life-time

THE life-time of the copper needles which were to be placed in orbit for the West Ford experiment is a matter of great concern. It is generally believed that the pressure of solar radiation (*not* drag) will bring the needles down in about seven years provided they are launched into a 'resonant' orbit (for example, a polar orbit at altitude 3,800 km.); for a good description of the resonance effect, see I. I. Shapiro and H. M. Jones[1]. These authors also show that if the semi-major axis of the orbit is changed by more than 150 km., the resonance would be destroyed and the life-time greatly increased.

My earlier article[2] discussed the problem of the drag of a charged body moving through a plasma and considered also just how a body acquires an electric charge in space. Although originally applied to micrometeors, this work can be carried over directly to the West Ford dipoles, which are copper needles of length 1·77 cm., diameter $2{\cdot}8 \times 10^{-3}$ cm., and mass 10^{-4} gm. I calculate a capacity C of $1{\cdot}5 \times 10^{-12}$ farad (my earlier article[2] adopts the wrong value of capacity, and, through force of circumstances, went to press before I could correct it; all further results there must be changed in proportion).

A more detailed examination of the charging effects for the particular conditions of the magnetosphere where the needles are put in orbit still leads to a potential of the needles close to the value adopted earlier. Its 'effective' value is $-3{\cdot}6$ V. Hence the nominal charge Z is $\sim 3 \times 10^6$ electrons.

A further detailed examination shows that within a small error it is permissible to replace the needle by a point charge when calculating its total interaction force with the magnetospheric plasma. Then with the use of the relations given earlier[2], the drag force acting on the needles turns out to be $F \sim 10^{-23} Z^2 n$ dyne, where n is the ion density $\sim 3 \times 10^3$ H^+ ions per cm.^{3} at the West Ford orbit. The resultant rate of decrease of the orbit radius is $\sim 3 \times 10^{-10} Z^2$ km. per annum. Hence the orbit shrinks at the rate of $dr/dt \sim 3 \times 10^3$ km. per annum.

The greatest uncertainty enters into the determination of the electric potential of the needle. I estimate it to be within $-7{\cdot}2$ to $-1{\cdot}8$ V., with a probability of more than 90 per cent. With these extreme limits the orbit would shrink at a rate as high as $1{\cdot}2 \times 10^4$ km. per annum (33 km. a day) or as low as 750 km. per annum.

It is clear, therefore, that not only will the drag spoil the resonance but also it will in all likelihood provide an upper limit for the life-time which amounts to a few months, possibly as much as three years.

However, one cannot entirely exclude the possibility that the root-mean-square potential is less than 0·25 V., leading to $dr/dt < 15$ km. per annum (in which case the radiation pressure resonance would be preserved and the life-time would be ~ 7 years); or that the root-mean-square potential is about 0·36 V., leading to $dr/dt \sim 30$ km. per annum, in which case the resonance would be destroyed and the life-time *increased* to nearly a century.

A detailed report has been prepared and will be published later.

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PHYSICS

Lattice-type Vibrations in Associated Liquids and the Origin of Anomalous Rayleigh Scattering

WHEN a sample is irradiated with monochromatic light of frequency ν_0 and the scattered light at right angles to this incident beam examined by means of a spectroscope, a line spectrum is observed containing besides the Rayleigh component ν_0, the Raman lines $\nu_0 \pm \Delta E_i/h$, where ΔE_i are the energy differences between stationary states of the sample. Although an arc having a relatively isolated line is normally used as the source of monochromatic light, all arcs do give rise to some continuum and this also appears in the spectrum as Rayleigh scattering. This continuum can be reduced by a carefully designed arc (such as the Toronto type[1] mercury arc), while the Rayleigh scattering is minimized by preparation of the sample for investigation in an optically clean condition. However, even under such favourable conditions some continuous scattering does occur, but the fact that it appears to depend on the nature of the scattering medium, extending less than 100 cm.$^{-1}$ from the exciting line in the case of unassociated liquids[2-4] and their corresponding solids and many hundreds of cm.$^{-1}$ in the case of highly associated liquids[5] and solids, would suggest that it is not Rayleigh scattering as is often thought. Its origin is, therefore, of interest, and it is the purpose of the present communication to discuss the possible sources of these anomalous Rayleigh 'wings'.

Phonon scattering, giving rise to a Brillouin spectrum, can be quickly ruled out as the major cause of this broadening since the velocity of sound in liquids and solids is of the order of 10^5–10^6 cm.sec.$^{-1}$, corresponding to frequency shifts of only ~ 1 cm.$^{-1}$ at 4,000 Å.

In unassociated liquids and their corresponding solids, rotation of the molecules should give rise to broad wings on either side of the exciting line, the broadness of these wings depending on the rotational constant and the temperature, and in general being less than 100 cm.$^{-1}$ for all but very light molecules. This is normally believed to be the predominant cause of the Rayleigh wings in these systems[2,4,6]. Although in associated liquids and solids rotation of molecules

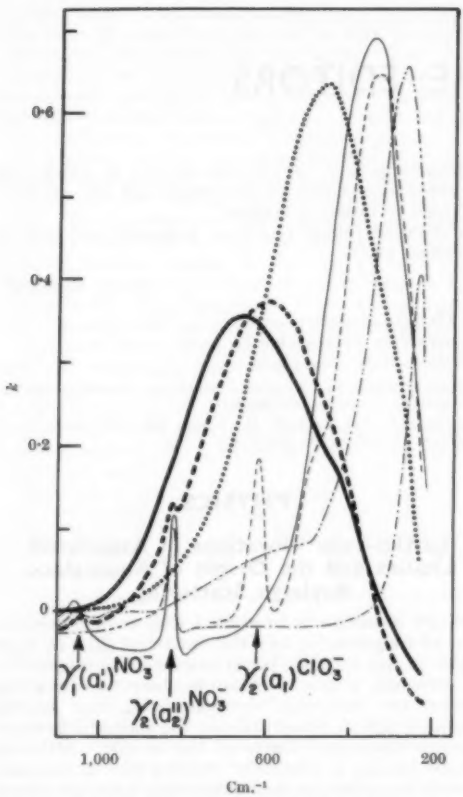

Fig. 1. The low-frequency infra-red spectra of some highly associated liquids. ——, Water (20° C.); – – –, $LiNO_3$–H_2O (5·92 *M* at 20° C.); ————, $LiNO_3$ (313° C.); ·—·—·—, $NaNO_3$ (347° C.); – – – –, $LiClO_3$ (250° C.); ·····, LiOH (490° C.); ·—·—·—, NaOH (350° C.). For both lithium and sodium hydroxides the ordinate scale should be multiplied by two

may still occur, the major cause of the Rayleigh wings probably arises from excitation of the optical modes of an associated or lattice-like structure. Such excitation of the optical modes may give rise to both a first- and a second-order Raman effect, the frequency maximum of the former corresponding roughly to that of the optical frequency observed in the infra-red, while the second-order Raman effect will extend out to approximately twice this frequency. Thus, solid sodium chloride, which has an infra-red absorption frequency[7] at 164 cm.$^{-1}$, exhibits a second-order Raman effect[8] extending out to 350–400 cm.$^{-1}$.

In the case of highly associated liquids, excitation of the optical branch of the quasi-lattice-like structure has not been reported previously. By using a one-angle reflexion technique[9] and an analysis due to Robinson and Price[10] the optical constants in the 200–1,000 cm.$^{-1}$ region have now been obtained for water, aqueous lithium nitrate, fused lithium nitrate, chlorate and hydroxide, and fused sodium nitrate and hydroxide, and the absorption spectra are shown in Fig. 1. The intense band in this low-frequency region can be assigned in every case to the excitation of the optical branch of a quasi-lattice, and the frequency of this band suggests that the Raman effect should show broad wings out to 400–900 cm.$^{-1}$ in fused salts and ~ 1,600 cm.$^{-1}$ in aqueous solutions, consistent with the known anomalous Rayleigh scattering in these systems.

It would appear of interest in the light of the above suggested origin of the anomalous Rayleigh wings to study this continuous scattering more fully, especially in the case of highly associated liquids. In this direction the 'laser', giving an intense monochromatic light source with no spurious arc continuum, should be ideal as a Raman source and should certainly clarify this question.

I wish to thank H. J. Bowlden for helpful discussion and criticism of the manuscript.

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The Balance of Energy in Nuclear Fission

Early experimental observations on the range of fission fragments in air[1] suggested that the kinetic energy of fragments of nearly equal mass formed in fission (that is, symmetrical fission) was less than that of fragments of different mass (that is, in unsymmetrical fission), and very recent measurements[2] have clearly confirmed this, and shown that the deficiency amounts to about 30 MeV. (32,210 micro-mass units, μM.U.). The conservation of energy in fission may be expressed by an equation of the form:

$$\left({}^{A}_{Z}M\right) = \left({}^{A_1}_{Z_1}M\right) + \left({}^{A_2}_{Z_2}M\right) + E_{12} \qquad (1)$$

where ${}^{A}_{Z}M$'s are the masses of the fissioning nucleus and of the fragments, 1 and 2, of mass number A and charge Z, and E_{12} is the energy released expressed in mass units. This last appears as the kinetic energy of the fragments together with the neutrons and γ-rays emitted in fission. It has been shown[3] that the neutrons and γ-rays associated with symmetrical fission are not markedly greater in energy than those associated with unsymmetrical fission, and it appears that the deficiency in the kinetic energy in question must be associated with an excess in the masses of the unstable fragments. The masses of the unstable fragments formed initially in fission are not known, however, and attempts to predict them from present empirical mass formulæ have not shown the expected mass excess[4].

Koenig, Mattauch and Wapstra have recently compiled a consistent set of all known stable and unstable masses[5]. In this communication it is shown that an extrapolation of these known masses suggests

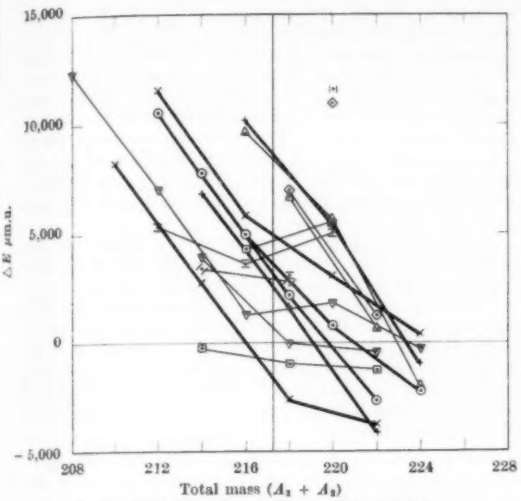

Fig. 1. Mass differences ($_{54}$Xe + $_{38}$Sr) − 2($_{46}$Pd)

	$A_2 - A_1$	$(A_2^* - A_2) - (A_1^* - A_1)$
⋈	34	− 9·25
⊡	36	− 7·25
▽	38	− 5·25
×	42	− 1·25
○	44	+ 0·75
+	46	+ 2·75
△	48	+ 4·75
◇	50	+ 6·75
⊣	52	+ 8·75

that symmetrical fission fragments have greater mass than unsymmetrical fragments.

For symmetrical fission, (*s*), equation 1 may be written:

$$\left(\begin{matrix}A\\Z\end{matrix}M\right) = 2\left(\begin{matrix}A_s\\Z_s\end{matrix}M\right) + E_s \qquad (2)$$

From equations (1) and (2):

$$\left(\begin{matrix}A_1\\Z_1\end{matrix}M\right) + \left(\begin{matrix}A_2\\Z_2\end{matrix}M\right) - 2\left(\begin{matrix}A_s\\Z_s\end{matrix}M\right) = E_s - E_{12} = \Delta E \qquad (3)$$

No protons are lost in fission and, therefore:

$$Z_1 + Z_2 = 2Z_s \qquad (4)$$

and because the average number of neutrons in symmetrical fission is about equal to that from unsymmetrical fission:

$$A_1 + A_2 = 2A_s \qquad (5)$$

In Fig. 1, ΔE from equation 3 is plotted against the total mass with $Z_s = 46$ (for symmetrical fission of $^{236}_{92}$U), and $Z_1 = 38$, $Z_2 = 54$, for all the known masses (on the carbon-12 scale) subject to condition (5). Lines connect points for mass differences $(A_2 - A_1)$ for combinations of nuclides which differ by equal numbers of neutrons. Heavy lines show mass differences for combinations of nuclides which are nearly equally deficient in neutrons with respect to their most stable isotopes, that is, when:

$$(A_2^* - A_2) \simeq (A_1^* - A_1) \qquad (6)$$

where * indicates the most stable mass. The graph suggests that extrapolation of the heavy lines to a total mass of 236 (the mass of the compound nucleus before fission or neutron emission) would lead to combinations of nuclides with a high negative value of ΔE.

In Fig. 2 ΔE for the other combinations of Z_1 and Z_2 have been plotted for highly unsymmetrical to symmetrical combinations of nuclides. Values of A have here been chosen subject to conditions (5) and (6). Fig. 2 suggests that linear extrapolation to higher masses would lead to high negative values of ΔE only for the elements lanthanum to tellurium and their complements (atomic numbers 57–52 and 35–40). If such an extrapolation is valid, it may be associated with the observations that the symmetrical fragments and also the highly unsymmetrical fragments are formed in low yield and with low kinetic energy,

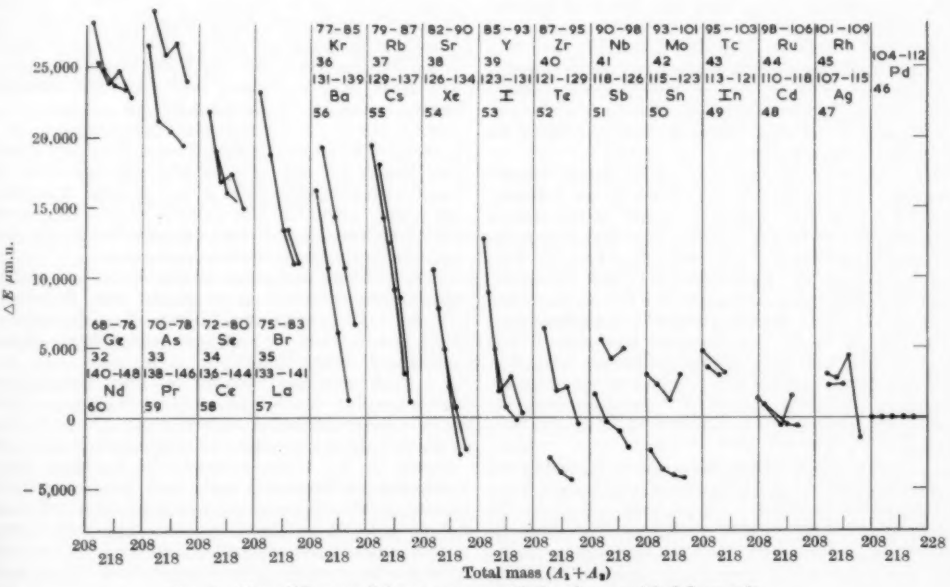

Fig. 2. Mass differences between unsymmetrical and symmetrical fragments

because there is less energy released in the formation of these fragments than in the formation of fragments with atomic numbers in the range shown. In addition, Fig. 1 gives a qualitative indication that the highest release of energy would occur in fission when nuclides are formed which are equally deficient in neutrons, and this is in accordance with the equal-charge displacement hypothesis for the distribution of charge in fission[6].

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ENGINEERING

Non-Linear Transmission Lines for Millimetre Wave Generation and Reception

The properties of non-linear transmission lines, containing, for example, ferroelectric material of the barium titanate class, have been examined in connexion with a computing system[1]. Their possible use as a variable capacity parametric amplifier element is obvious, and it has been shown recently that an electromagnetic shock-wave can be propagated[2]. This effect occurs when a signal of large amplitude is injected into the line. The high-field part of the wave travels at a greater speed than the low-field part so that a sine wave becomes a saw-tooth. After a certain time this has a vertical leading edge analogous to a shock-wave in a gas. Such a wave is rich in harmonics and a number of lines, driven with power derived from the same microwave magnetron and with outputs recombined through phase shifters, could be an efficient harmonic generator.

A less obvious method of generating higher frequencies with a non-linear line is as follows: Suppose a low-level microwave signal is fed into a wave-guide filled with ferroelectric, and that this guide is constructed so that it is possible to apply a d.c. bias voltage to change the permittivity of the dielectric. Suppose the signal pulse duration is for a certain time and that during this time the bias is changed so that the velocity of wave propagation increases. The emergent signal will then occupy a shorter time, but as it contains an unchanged number of oscillations the frequency will be increased. Also as energy is conserved the power will increase, if the losses can be neglected.

The contrary case, where the wave is slowed down during the pulse so that the pulse is stretched in time and reduced in frequency, is also of interest. In principle this would permit millimetre waves to be converted to a more easily detected frequency. These effects are large if the switching time of the bias voltage is of the order of the time taken by a wave to pass along the transmission line, which imposes some technical limitations.

However, the chief difficulty at present is in the ferroelectric material. Measurements at 10-cm. wavelength of permittivity variation with bias have shown that the effect is still appreciable in a barium–strontium titanate ceramic[3]. Measurements at 3-cm. wavelength showed that relative permittivities of several thousand are still obtained[4]. There appears to be no published work on these materials at higher frequencies and with d.c. bias applied.

I hope to make some measurements along the lines indicated in this communication at shorter wavelengths than 3 cm. As I have no facilities at present for material manufacture, these measurements will be initially with barium–strontium titanate supplied by an industrial research laboratory. I would be interested to hear from any organization producing titanates or the newer ferroelectric materials which would care to collaborate in a programme of millimetre wave measurements.

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GEOLOGY

Carbonatitic Lava from Fort Portal Area in Western Uganda

In an excellent paper Holmes[1] quotes chemical analyses of two spectacular carbonated lavas, one from the Katwe region, the other from Kalyango volcano near Fort Portal. In earlier work, Holmes and Harwood[2] described a similar carbonated lava from Lake Kyekora, also near Fort Portal. Present examination of a vesicular lava of high carbonate content poured out from Kalyango volcano shows close geochemical affinities with intrusive carbonatites. It must be emphasized that the lavas examined are fresh and unaltered and that the carbonated nature cannot be attributed to alteration after solidification. A proposed investigation of the numerous lavas in the Fort Portal field may elucidate the formation not only of the characteristic ultrabasic potassic volcanics of south-west Uganda and adjoining parts of the Congo, the petrogenesis of which Holmes has presented so admirably, but also the genesis of the carbonatites themselves.

The field relationships of the Kalyango and other similar 'carbonated' lavas have been described by Combe[3]. Extrusive equivalents of carbonatites are rare, one other occurrence being from Kerimasi volcano in Tanganyika[4].

A mineralogical, chemical and spectrographic investigation of the lava from Kalyango volcano shows the following characteristics: The rock is light-grey in colour, variably vesicular with phenocrysts of biotite up to 1 cm. across. It contains frequent inclusions of basement rocks and occasional rounded, possibly eclogitic fragments consisting of a dark-green clinopyroxene and pink garnet. The dense groundmass is powdered with magnetite and the vesicles are lined with a crust of small crystals of calcite and are often filled with spherules and rosettes of fibrous

Table 1. CHEMICAL ANALYSES OF CARBONATITIC LAVA FROM UGANDA

	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	FeO	MnO	MgO	CaO	Na_2O	K_2O	H_2O+	H_2O-	P_2O_5	CO_2	Total
1.	12·99	1·74	3·03	7·93	4·44	0·40	8·55	35·97	0·73	0·20	3·45	1·65	3·32	14·79	100·62
2.	13·81	1·96	3·20	8·05	4·33	0·44	8·65	35·42	1·03	0·17	3·76	2·89	3·57	11·27	100·05

1. Vesicular carbonatitic lava from a flow, Kalyango Volcano, Fort Portal. Total includes SrO = 0·63, BaO = 0·15, S = 0·35, F = 0·3.

2. Vesicular carbonatitic lava rich in zeolites, same locality. Total includes SrO = 0·60, BaO = 0·20, S = 0·40, F = 0·3.
(Analysis by O. von Knorring.)

zeolites. Under the microscope the ground-mass has a felt-like appearance containing minute grains of pyroxene, olivine, biotite, magnetite, some ilmenite, apatite and calcite. The larger clinopyroxene and biotite grains are seen to be corroded and the latter are often replaced by calcite along the cleavage. In polished sections magnetite and pyrite are the most conspicuous ore minerals. A partial analysis of magnetite showed some 11 per cent titanium dioxide; in reflected light, however, no exsolution lamellæ of ilmenite were seen. From partial analysis, the biotite is a magnesia-rich variety with a high titania content, and rather similar in composition to a biotite recorded by Combe and Holmes[5].

Among the major constituents of the lava (Table 1) silica and alumina are extremely low compared with other ultrabasic igneous rocks. Low silica contents of this kind are known in certain carbonate-rich alnöitic dyke rocks, for example in the so-called aillikite from Aillik Point in Labrador[6,7]. The calcium content is high, and most of the calcium is combined in silicates. The carbon dioxide content appears to be variable, and therefore lavas with even higher carbonate contents are possible, as shown by the almost pure carbonatite analysis by Holmes. Phosphorus, usually a minor constituent, is extremely high in the lava.

The pattern of the minor and trace elements is typical of carbonatite rocks. A feature common in some of these rocks is the dominance of strontium over barium, in addition to high lanthanum and niobium contents. In carbonatites the latter element often forms pyrochlore; in this case, however, a black, opaque perovskite in cubes 0·01–0·05 mm. across is believed to be the principal carrier of niobium.

The spectrographic results of the lava (Table 2) show, on one hand, similarities with rocks of the Toro-Ankole field, for example the katungites, and on the other close resemblance to carbonatites as determined by Higazy[8].

Table 2. SPECTROGRAPHIC ANALYSES OF CARBONATITIC LAVA FROM UGANDA (P.P.M.)

	Li	Sc	V	Cr	Co	Ni	Ga	Y	Zr	Nb	Mo	La	Pb
1.	19	13	200	35	45	45	14	65	550	550	7	600	40
2.	14	—	250	30	25	50	12	85	800	550	7	600	45

(Analysis by Miss J. M. Rooke.)

A carbonatite magma, containing water and other volatiles like phosphorus, fluorine and sulphur, can effectively react with silicate rocks during intrusion through the sialic crust to produce the characteristic suite of rocks encountered among carbonatites and associated alkaline complexes. As a result of this interaction a part of the alkalis are released and may migrate to induce metasomatism in the country rock, as often expressed in the well-known phenomenon of fenitization. Selective concentration of alkalis in addition to differentiation may, moreover, result in sodium- and potassium-rich rocks.

The present carbonatitic lava provides further evidence for the magmatic nature of carbonatites and supports the hypothesis advanced by Holmes, that a carbonatite (magma or fluid) is instrumental in producing the remarkable group of volcanic rocks in south-west Uganda and the Congo.

We are indebted to Prof. W. Q. Kennedy for his interest in the work, Mr. A. Cawley, director of the Uganda Geological Survey, for permission to publish this communication, and to Miss J. M. Rooke for the spectrographic analyses.

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CRYSTALLOGRAPHY

Structure of Molybdenum Sesquisulphide

THE existence of molybdenum sesquisulphide, Mo_2S_3, as a distinct phase has been in doubt for a considerable time. It was only in 1955 that McCabe[1] convincingly demonstrated the existence of the phase and obtained its X-ray powder pattern. An attempt to index the powder pattern has been made by Stubbles and Richardson[2]; they tentatively propose a tetragonal unit cell with $c = 10{\cdot}9$ Å.; $c/a = 1{\cdot}27$.

I have undertaken a new investigation of Mo_2S_3 by X-ray powder methods, using a Philips diffractometer. It was found that the true symmetry of the phase is monoclinic; the unit-cell dimensions are: $a = 8{\cdot}633_5$ Å.; $b = 3{\cdot}208$ Å.; $c = 6{\cdot}092$ Å.; $\beta = 102{\cdot}43°$. (Evidently, Stubbles and Richardson[2] were misled by the coincidence that a is almost exactly equal to $\sqrt{2} \times c$.) With a cell content of Mo_4S_6 the density is calculated as 5·806 gm./cm.^{3}.

The lines 010 and 030 are not observable in the powder diagrams, while 020 and 040 are very strong. Furthermore, the intensity sequence of the reflexions $h0l$ is found to be the same as that of $h2l$ and $h4l$; the same is true of $h1l$ and $h3l$. This indicates that Mo_2S_3 crystallizes in the space-group $P2_1/m$, with all atoms in the special position $2(e)$: $\pm (x, \frac{1}{4}, z)$. The intensity relations mentioned here made it possible to divide the intensities of the (not too numerous) pairs of coinciding powder lines. Thus, a complete set of experimental structure factors covering the copper-$K\alpha$ sphere was obtained and the structure could be determined by the usual methods.

A three-dimensional electron-density map is shown in Fig. 1. The sulphur atoms form a somewhat distorted close-packed lattice with layer sequence *chh*. The metal atoms are inserted in part of the octahedral holes of this lattice. The holes between neighbouring h-layers are completely occupied, while only half the

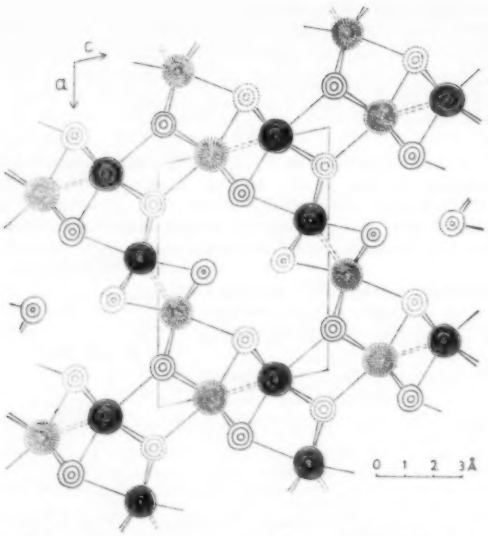

Fig. 1. Electron density of Mo_2S_3. Contours are at approximately 10 el./Å.^{3}. Full-line contours refer to atoms at $y = \frac{1}{2}$; broken contours to atoms at $y = \frac{3}{4}$. Mo—S bonds are indicated by full lines, the Mo—Mo chains by broken lines. The outlines of the unit cell are also indicated

holes between the other pairs of neighbouring sulphur layers are filled. Thus, the sequence of layers in the *a* direction is: . . S Mo S $Mo_{1/2}$ S $Mo_{1/2}$ S Mo S . . .

The molybdenum atoms are not in the centres of the sulphur octahedra; they are shifted by 0·32 Å. in the direction of one of the octahedron faces, in such a way that zig-zag Mo—Mo chains running in the *b* direction are formed. Within these chains the Mo—Mo distances are 2·85 Å., which is slightly longer than in the metal itself. Each molybdenum atom has three sulphur neighbours at distances of 2·36 Å. and three more at 2·57 Å. In MoS_2, where the metal is in trigonal-prismatic surroundings[3], the Mo—S distances are 2·35 Å.

I wish to thank the Climax Molybdenum Company of Michigan for a gift of pure Mo_2S_3. The assistance of Dr. C. F. van Bruggen and Mr. H. Schurer in this investigation is gratefully acknowledged.

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CHEMISTRY

Incorporation of Trivalent Cations in Crystalline Potassium Chloride

Divalent metal halides such as calcium chloride, dissolved in alkali halide crystals, increase the ionic conductivity[1] and give rise to additional bands (the *Z* bands) in the absorption spectra of coloured alkali halides[2]. For some time there has been speculation concerning the possibility of incorporating trivalent cations and the effect of such additions on electrical and optical properties.

Little experimental information is at hand. Hayes and others[3–5] suggested, on the basis of paramagnetic resonance studies, that sodium fluoride grown from a melt with added chromium chloride contained the chromium as Cr^{++}; on irradiation, reduction to Cr^{+} was thought to occur. Schnerb and Bloch[6] found that potassium chloride crystals grown from aqueous solution containing added ferric chloride were opaque. Schulze[7] considered the dependence of the ratio of Sm^{++} to Sm^{+++} in potassium chloride, grown from a melt containing samarium chloride, on the pressure of chlorine over the melt.

Lidiard[8] showed that if the ionization potential associated with the removal of an electron from an atom or ion to yield a given oxidation state were greater than about 22 V., the element would not exist as a substitutional impurity in solid potassium chloride in the given oxidation state but in the lower one. Of elements having the stable + 3 state the third ionization potentials of which are known, only yttrium, lanthanum and cerium have potentials somewhat less than 22 V. The third ionization potentials of other rare earths are unknown: however, these elements, being similar to cerium, may dissolve in potassium chloride in the trivalent state. The boiling points of the lanthanon trihalides are all above 1,475°, so that evaporation from molten potassium chloride is negligible.

We have attempted to prepare single crystals of potassium chloride containing lanthanum, yttrium, gadolinium, dysprosium, samarium and neodymium; we have performed chemical analyses, examined the ionic conductivity, and studied the absorption spectra after additive and radiative coloration of a number of samples. Fifteen crystals were doped with gadolinium, four each with dysprosium and samarium, three each with lanthanum and yttrium, and one with neodymium.

Each of the anhydrous trihalides was prepared by drying the hydrate in a stream of dry hydrochloric acid mixed with nitrogen; the product was stored in a sealed vial and introduced into molten potassium chloride by breaking the vial under the surface of the melt. The composition of the melt was usually about 1 per cent in the added trihalide. Single crystals were grown by Czochralski's method. In the presence of air, the melt gradually became turbid due to formation of oxyhalide of the added element; some crystals were grown in an atmosphere of dry hydrochloric acid, which prevented this occurrence. The crystals frequently contained milky regions due to precipitation of the trihalide as a separate colloidal phase on cooling from the temperature of growth. Viewed along the axis of withdrawal of the seed-crystal, the white portion usually formed an X. The reason for this interesting observation is as yet unknown.

The crystals were analysed for rare earth elements (we include here yttrium and lanthanum) spectrophotometrically by means of the alizarin red *S* method due to Rhinehart[9]. Ionic conductivity was determined by means of a vacuum-tube volt-ohmmeter (supplied by the Precision Apparatus Co. Inc.), using platinum electrodes pressed against the faces of the sample crystal. Crystals were coloured additively by a method previously described[10]; a copper-target tube operated at 35 kV. was used for X-ray coloration.

The results of the chemical analysis clearly separated the crystal samples into two quite distinct

categories. Those containing precipitated material as evidenced by a milky appearance contained from 0·90 to 133 × 10^{17} rare earth ions/cm.^{3}. The former was doped with yttrium, the latter with gadolinium. Clear sections of crystal in no case contained more than 0·70 × 10^{17} cm.$^{-3}$, and in most cases the rare earth content was less than 0·50 × 10^{17} cm.$^{-3}$, the lower limit of detection. Dr. W. Hayes kindly examined clear sections of the gadolinium- and dysprosium-doped crystals using paramagnetic resonance, and reported no observable resonances attributable to trivalent ions. In the case of gadolinium, the sensitivity was such that 0·01 × 10^{17} trivalent ions/cm.^{3} would have been observed.

The ionic conductivity, and its temperature dependence, of the clear sections of all crystals was indistinguishable from that of crystals grown from recrystallized reagent-grade potassium chloride without added trihalide. Extrinsic conductivity of crystals containing colloidal precipitate was higher; calculations based on the data of Kelting and Witt, for a sample containing 28·5 × 10^{17} gadolinium ions/cm.^{3}, indicated that the conductivity at 370° corresponded to that due to 2 × 10^{17} trivalent ions/cm.^{3} or 4 × 10^{17} divalent ions/cm.^{3}. However, some of the additional conductivity may arise from irregularities in the crystal caused by the strain in the neighbourhood of the colloidal particles, and in view of the conductivity measurements on clear sections and the measurements of Hayes, we must conclude that the solubility of the trivalent cations at room temperature is below the limits of detection used here. The solubility of the rare earth elements in the divalent state may be somewhat greater, but in any event not greater than about 4 × 10^{17} ions/cm.^{3} at 370°. Experiments with radioactive tracers would be useful.

The absorption spectrum of additivity-coloured crystals doped with the trivalent metal halides exhibited no new bands resolvable by observation at 90° K. Crystals containing colloidal precipitate could not be coloured by treatment with potassium vapour. We presume that under the stringent reducing conditions, the rare earth element was reduced to metal; in fact, metallic specks were formed which were soluble in concentrated hydrochloric acid to give solutions yielding a strongly positive test for rare earth element with alizarin red *S*.

On the other hand, such crystals were coloured by X-irradiation. A broad band with a maximum at 325 mμ was observed in several crystals containing dysprosium and samarium in precipitated form. This band has not been obtained at room temperature in the case of undoped crystals, although Dorendorf[11] observed two small bands in this region in nominally pure potassium chloride irradiated with X-rays at −180°, which he designated V_6 and V_7. Either the rare earth impurity causes the band or enhances the stability of Dorendorf's bands at room temperature.

We conclude that the solubility of these trivalent cations is too small for them to exert any pronounced effects on the properties of potassium chloride crystals of ordinary purity. Their incorporation in potassium chloride of exceptional purity would probably be observable through their effect on extrinsic conductivity.

The assistance of F. Block and G. Robidart in obtaining and preparing the rare earth halides and that of Dr. W. Hayes, who measured the paramagnetic resonance absorption of several samples, is gratefully acknowledged.

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Purification of Hydrogen Isotopes by Self-Displacement Chromatography at Low Temperatures

Gluekauf[1] has shown that protium and deuterium can be separated by displacement gas chromatography using columns of palladium black dispersed on asbestos. The most effective method was stated to be that of self-displacement, whereby the columns were initially charged at room temperature with a mixture of isotopes and then heated from the inlet end, progressively desorbing the gases. Because protium was more strongly adsorbed than deuterium, it functioned as the displacing gas in a displacement chromatography system, and the gases were desorbed as a band of deuterium followed by a band of protium. We have discovered that a similar method, using a column containing Molecular Sieve 5*A* initially at liquid-nitrogen temperature, results in an excellent separation of hydrogen isotopes with the difference that in this case the leading band consists of protium.

A column was prepared from two lengths of copper tubing each 10-ft. long by 3/16 in. bore, filling them with Molecular Sieve 5*A* of particle size 30–44 B.S.S. mesh and then activating the sieve by heating to 350° C. *in vacuo*. Each section was coiled into a helix of 4 in. diameter and they were joined together in series at a valve, which, with valves at the inlet and outlet ends, permitted isolation of both sections. The sections could be immersed separately in liquid nitrogen contained in Dewar flasks. Initially, the column was either evacuated, or filled with helium or deuterium at room temperature and isolated. It was then cooled and a measured volume of a hydrogen isotope mixture admitted to the first section of the column. The valve between the two sections was opened, and the column was slowly and progressively warmed from the inlet to the outlet end by successive lowering of the two Dewar flasks. The mixture absorbed at the inlet was thus caused to move along the column, and two bands of the pure isotopes developed. When the pressure inside the columns equalled atmospheric, the outlet of the second column was opened to a monitoring katharometer and the bands of displaced gases collected. The order of displacement was helium (if initially present), protium, a mixed fraction of both isotopes and finally pure deuterium. The trace obtained from the monitoring katharometer during the separation of a 10 per cent protium in deuterium mixture is shown

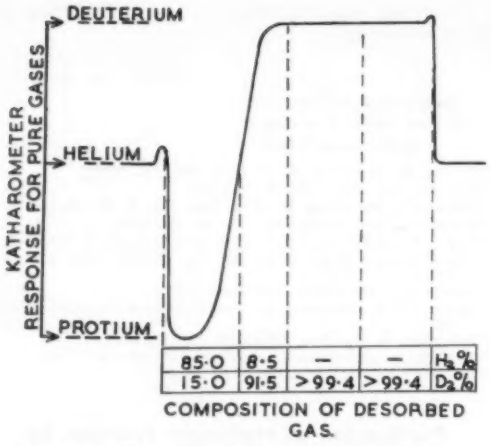

Fig. 1. Output of monitoring katharometer during self-displacement chromatography of a 10 per cent protium in deuterium mixture

in Fig. 1, the fractions collected being shown by the dotted lines. The fractions were analysed by gas elution chromatography[2].

If the original mixture is deficient in deuterium (the displacing gas) there may not be sufficient present to complete the displacement of the protium and in this case the purity of the deuterium band will not be as high. This was found to occur (Table 1), but even for a mixture containing only 25 per cent deuterium a considerable purification of deuterium was possible.

Table 1

Composition of original mixture	Fractions collected Composition H_2 (per cent)	D_2 (per cent)	Vol. (ml.)
	85	15	33·0
	8·5	91·5	40·2
90 per cent D_2, 10 per cent H_2	—	>99·4	121
	—	>99·4	101
	>99·4	—	110
	99·4	0·6	79
25 per cent D_2, 75 per cent H_2	27·3	72·7	42
	10·6	89·4	50

The process is capable of operation on a larger scale and charges of 2·5 litres in volume have been processed using a first section of copper tubing 15 in. in length by 3/4 in. bore, packed with 1/16 in. pellets of Molecular Sieve 5*A* and the second of the same dimensions as used previously. The second section was effective as a front sharpener as well as contributing to band formation, by reducing the diffusion effects inherent in wider bore columns and hence the volume of the mixed band between the two fractions. Results with the larger columns were comparable to those with the small columns. For example, from 2·0 litres of mixture containing 8 per cent protium, 1·7 litres of deuterium containing 1 per cent or less of protium were obtained in a single run over a period of 1 hr.

The adsorptive capacity of the Sieve is 43·5 ml. of protium/gm. and 47·5 ml. of deuterium/gm. With such relatively high capacities, a laboratory-scale apparatus may readily be constructed which is capable of purifying several litres of hydrogen isotope mixture per hour. The process can be made repetitive, for after the first run the column requires no further preparation, the deuterium remaining being re-adsorbed on to the first section by cooling before the next charge is admitted.

With the limitation that the columns had to be filled initially with helium at atmospheric pressure, columns containing alumina impregnated with ferric oxide were found to operate in a similar manner, but their adsorptive capacity was less. Self-displacement chromatography using columns of pure alumina would appear to offer a method of separation of quantities of the spin isomers of either protium or deuterium as it has been shown they may be separated by elution chromatography on such a column[3].

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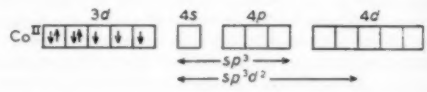
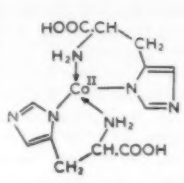
Structure of the Oxygen-carrying Cobalt (II) Histidine Complex

Bis-histidinato cobalt (II) is one of the few synthetic compounds which can combine reversibly with molecular oxygen. Hearon *et al.*[1] obtained a value of 4·44 Bohr magnetons (B.M.) for its effective magnetic moment at room temperature in aqueous solution, and showed that on oxygenation it became almost diamagnetic with the absorption of one oxygen molecule for two cobalt atoms. For three unpaired electrons the spin-only moment would be 3·88 B.M., and the considerably larger experimental value obtained in this case was ascribed to incomplete quenching of the orbital contribution to the moment. From the magnetic measurements Hearon assigned a tetrahedral structure (sp^3 hybridization), I, to the complex:

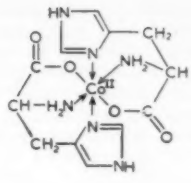


in which the cobalt atom was co-ordinated to the α-amino group and an imidazole nitrogen atom of each histidine molecule. However, histidine can behave as a tridentate ligand, and an octahedral configuration, II, involving co-ordination of ionized carboxyl groups is not unlikely. Hearon considered this to be only a secondary possibility.

Because of the differing orbital contributions, tetrahedral and octahedral cobalt (II) complexes generally have effective magnetic moments at room temperature in the ranges 4·3–4·7 and 4·8–5·6 B.M. respectively[2]. This would appear to confirm the tetrahedral structure of the complex, but Hearon has reported its aqueous solution to be pink, typical of octahedral rather than the commonly blue tetrahedral complexes.



(I)



(II)

We have prepared solid *bis*-histidinato cobalt (II) in oxygen-free conditions, using an apparatus already described[3], by concentrating the solution obtained by dissolving cobalt (II) hydroxide in an aqueous solution of histidine—the method used by Jaselskis[4]. Our analyses agree with his formulation of the compound as the monohydrate. The solid is pink with an effective moment of 4·81 B.M. at 291° K.; it obeys the Curie–Weiss law between 80 and 300° K., and $\theta = 17°$; these values are comparable with those obtained for other spin-free, octahedral cobalt (II) complexes by Figgis and Nyholm[2b]. The variation with temperature of molar susceptibility χ_M and effective moment $\mu_{eff.}$ is shown in Table 1.

Table 1

T° K.	86·2	116·3	147·8	176·2	204·7	232·1
$\chi_M \times 10^6$	29,750	22,850	18,630	15,900	13,890	12,320
$\mu_{eff.}$	4·55	4·63	4·71	4·75	4·79	4·80
T° K.	261·5	291·0	305·3	322·2		
$\chi_M \times 10^6$	10,910	9,833	9,453	8,956		
$\mu_{eff.}$	4·80	4·81	4·82	4·83		

The magnetic moment of the compound re-dissolved in water was 5·16 B.M. at 297·2° K., much higher than that recorded by Hearon. The measurements were repeated on a solution of the complex prepared by mixing aqueous solutions of histidine and a cobalt salt, and a value of 5·13 B.M. was obtained. This method was used by Hearon, but he allowed momentary exposure to the atmosphere during the filling of the susceptibility tube. There is some inconsistency in the concentrations recorded by Hearon for his solutions and we believe that either crystallization or partial oxidation of the compound may account for the low moment obtained. Our values of moment from the solution measurements are well within the range expected for octahedral spin-free cobalt (II) complexes, and agree with the value of 4·97 B.M. reported by Michaelis[5] for the complex in solution.

The visible absorption spectrum of the complex in aqueous solution[1] is very similar to the spectra of octahedrally co-ordinated cobalt (II) complexes[6] and shows a weak band at 486 mμ ($\varepsilon = 18{\cdot}4$). The reflectance spectrum of the solid has a peak at the same wave-length.

In the infra-red spectrum of the cobalt histidine complex obtained in a 'Nujol' mull a broad, intense band appears at 1,587 cm.$^{-1}$, which is unchanged on deuteration. Since it is well below the 1,700 cm.$^{-1}$ region where non-ionized carboxyl groups absorb[7], it must arise from the asymmetrical stretching vibration of ionized and probably co-ordinated carboxyl groups. Consequently, structure I, which necessitates uncoordinated non-ionized carboxyl groups, cannot be correct.

These measurements show that the cobalt atom is in an octahedral environment and are in agreement with the recent work of Leberman and Rabin[8], who have deduced from potentiometric titration data that all three available donor groups of the histidine molecules are co-ordinated to the metal atom in complexes with cobalt (II), nickel (II), zinc (II), and probably cadmium (II).

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Some Tetrahydropyranyl Derivatives of Glycine, Serine and Urea

The provision of model compounds containing amino-acids or their analogues attached to sugars is of importance in evaluating the stability of the numerous types of carbohydrate-peptide linkages theoretically possible in mucoproteins. The ability of 2,3-dihydropyran to condense with —OH, —SH, and —NH_2 groupings suggested a route to the glycosidically linked model compounds. The tetrahydropyran-2-yl derivatives so formed had the added advantage that the lability of the glycosidic linkage could be investigated without attendant complications such as acyl migration under alkaline conditions or Amadori re-arrangement catalysed by acid which might ensue in the presence of a hydroxyl substituent at C–2 of the pyran ring.

In the condensation between 2,3-dihydropyran and *N*-benzylsulphonyl-serine (DL-mixture) the tetrahydropyran-2-yl residue was introduced solely at the hydroxymethyl grouping; no evidence for reaction with the acidic hydroxyl was detected. *N*-Benzylsulphonyl-serine (9·7 gm.) in ether-ethyl acetate (1 : 1 v/v) was stirred at ambient temperature for 15 days with 2,3-dihydropyran (9·7 gm.) in the presence of two drops of concentrated hydrochloric acid. After neutralization (silver carbonate), the solution was evaporated and the residue extracted successively with water and ethyl acetate. The latter extract yielded crystalline *O*-tetrahydropyran-2-yl-*N*-benzylsulphonyl-serine (10·4 gm.), m.p. 130–132°, after five recrystallizations from ethyl acetate. The optically inactive product contained C, 52·5; H, 5·8; S, 9·55 per cent and had an equivalent weight of 349 by titration; $C_{15}H_{21}NSO_6$ requires C, 52·4; H, 6·1; S, 9·3 per cent and an equivalent weight of 343. Addition of aqueous 0·5 *N* silver nitrate to a cooled (0°) methanolic solution of the serine compound precipitated the crystalline silver salt. Found : C, 40·8; H, 4·5; N, 3·1; Ag, 23·4 per cent; $C_{15}H_{20}O_6NSAg$ requires C, 40·0; H, 4·5; N, 3·1; Ag, 23·95 per cent. Under similar conditions *S*-tetrahydropyran-2-yl-L-cysteine yields the silver mercaptide of cysteine[1].

Preparation of the *N*-glycoside adducts was rendered difficult by their instability. An aqueous solution of glycine (10 gm.), tetrahydropyran-2-ol (5-hydroxypentanal) (13·4 gm.) and sodium bicarbonate (12 gm.) was stirred at room temperature for 90 hr. The residue obtained on evaporation (below

40° C.) was kept in a vacuum desiccator over phosphorus pentoxide for 3 days and further dried for 1 hr. at 63° C. Thereafter, the residue (4·7 gm.) was extracted with dry methanol and the crude sodium salt of *N*-tetrahydropyran-2-yl glycine precipitated with ether. After six such treatments a relatively pure but unstable amorphous product was obtained giving a positive Erhlich reaction[2] (without acetylacetone). On paper chromatograms irrigated with butan-1-ol/ethanol/water (4 : 1 : 5) it decomposed into a mixture of tetrahydropyran-2-ol (detected with a 2,4-dinitrophenylhydrazine spray[3]) and sodium glycinate (revealed by ninhydrin[4]). The corresponding ethyl and benzyl esters of *N*-tetrahydropyran-2-yl glycine were more stable and moved as chromatographically homogeneous components with R_F 0·89 and 0·90 respectively (cf. glycine ethyl ester R_F 0·4) as revealed by chlorine–potassium iodide treatment[5]. Kaverzneva and Kalis[6] have shown that the sodium salt of *N*-D-glucosylglycine is readily hydrolysed in aqueous solution at room temperature. Other workers[7] have demonstrated that *N*-D-glucosylglycine ethyl ester undergoes slow hydrolysis in aqueous solution at room temperature and is rapidly and quantitatively hydrolysed at 90° C. The pyranyl derivatives were prepared by reaction of the appropriate ester and dihydropyran for 17–20 days at room temperature in dimethylformamide (ethyl ester) or ether (benzyl ester; anhydrous magnesium sulphate added) containing a trace of mineral acid. A mixture of dihydropyran (2·1 ml.) and urea (0·6 gm.) in dimethylformamide (30 ml.) containing a trace of concentrated hydrochloric acid gave, after 96 hr. at 17° C., two products with R_F 0·70 and 0·93 (butan-1-ol–ethanol–water). The mixture was taken up in water (30 ml.), extracted with chloroform (6 × 50 ml.) which was back-washed with water (2 × 50 ml.). The dried chloroform solution contained the more mobile component which, after re-crystallization from acetone (twice), chloroform (twice) and ethanol (six times), yielded pure *NN'*-di-tetrahydropyran-2-yl-urea, m.p. 211–212° C., dropping to 201–202° C. after standing for 24 hr. The freshly isolated compound contained C, 57·9; H, 8·5; N, 12·3 per cent; $C_{11}H_{20}O_3N_2$ requires C, 57·9; H, 8·8; N, 12·3 per cent. The aqueous layer obtained after chloroform extraction of another urea-dihydropyran condensation was freeze-dried and separated on a cellulose column eluted with butan-1-ol/ethanol/water (4 : 1 : 5). The *N*-tetrahydropyran-2-yl-urea fraction (R_F 0·70) so obtained was repeatedly recrystallized from chloroform and ethanol to yield the pure compound, m.p. 187–188° C. (melting at 178–179° after 24 hr.). Found : C, 50·0; H, 8·3; N, 19·4 per cent; $C_6H_{12}O_2N_2$ requires C, 50·0; H, 8·3; N, 19·4 per cent. Hydrolysis of both pyranyl ethers at 100° with 'Zeo-Karb 225' (H^+) afforded urea and tetrahydro-pyran-2-ol (characterized as its 2,4-dinitrophenylhydrazone, m.p. 107–109° C. [8]). Benn and Jones[9] have characterized the products of the acid-catalysed condensation of D-glucose and urea as *N*-β-D-glucopyranosyl- and *NN'*-di-β-D-glucopyranosyl-urea.

The relative stability of the tetrahydropyranyl derivatives was determined by hydrolysis of an aliquot (8–10 mgm.) in 1 ml. of aqueous acid or alkali, neutralization and estimation of the tetrahydropyran-2-ol liberated using alkaline hypoiodite[10]. Control experiments showed that the original compound was stable under the conditions used in estimation. *N*-Tetrahydropyran-2-yl-urea was more stable under alkaline conditions [0·1 *N* sodium hydroxide for 15 min., percentage hydrolysis 30 (80° C.), 57 (100° C.)] than acid conditions [0·01 *N* hydrochloric acid for 15 min., percentage hydrolysis 11 (40° C.), 67 (60° C.), 87 (80° C.), 100 (100° C.)]. A similar stability was exhibited by *NN'*-di-tetrahydropyran-2-yl-urea [0·01 *N* hydrochloric acid for 15 min., percentage hydrolysis, 97 (100° C.) : 0·1 *N* sodium hydroxide for 15 min., percentage hydrolysis, 61·5 (100° C.)] *N*-Tetrahydropyran-2-yl-glycine decomposed in water within a few minutes, but was stable in ethereal suspension at room temperature. The stability of *O*-tetrahydropyran-2-yl-*N*-benzylsulphonylserine was not much different under corresponding acid [0·01 *N* hydrochloric acid for 15 min., percentage hydrolysis, 25 (40° C.), 71 (60° C.), 92 (80° C.), 100 (100° C.)] and alkaline conditions [0·01 *N* sodium hydroxide for 15 min., percentage hydrolysis, 37 (50° C.), 67 (60° C.), 100 (100° C.)].

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Carcinogenicity of the Methyl-1,2-Benzanthracenes and their Derivatives

A number of theories have recently been put forward to account for the carcinogenic activity of the polycyclic aromatic hydrocarbons; in their present form, the majority of these theories do not provide a rationale for the fact that a number of chemically dissimilar carcinogens appear to operate through some common protein deletion principle[1]. Previous descriptions of a possible mechanism of carcinogenesis[2–4] have pointed out, however, that, in principle at least, the unifying mechanism by which such carcinogens act may be their formation of charge-transfer complexes with proteins; we are now able to discuss the activities of the methyl-1,2-benzanthracenes and the mono-fluoro-10-methyl-1,2-benzanthracenes (Fig. 1) in such terms.

The calculation of the electron distribution in these molecules has been performed within the framework of the usual linear combination of atomic orbitals-molecular orbital (L.C.A.O.–M.O.) method, where we describe the molecule by specifying which molecular orbitals are allowed for the π electrons and what are their energies. The orbital energies are given in the form:

$$E_i = \alpha + k_i\gamma$$

α and γ being the usual Coulomb and resonance integrals of the Wheland approximation. The difference in energy between two levels i and j is quite simply:

$$\Delta E_{ij} = (k_i - k_j)\gamma$$

and is therefore independent of α. The values of the coefficients $(k_i - k_j)$ are listed in Table 1.

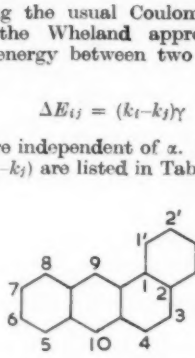

Fig. 1. Numbering of the ring positions in 1,2-benzanthracene

In the case of the aromatic hydrocarbons, the criterion for activity was established[2,3] as:

$$\Delta E_{1,\bar{2}} \approx 3{\cdot}24 \pm 0{\cdot}12\ \text{eV}.$$

This energetic criterion was taken as implying that for a π complex to describe the transition state of the hydrocarbon–protein interaction, matching of energy levels in the complex was necessary; the highest filled orbital of the hydrocarbon coincided with the lowest filled band of the protein while the second unfilled level acted as a resonating level for conversion of electrons from the valence band to the lowest unfilled level of the carcinogen. An inspection of Table 1 now shows that the active range for the methyl-1,2-benzanthracenes and the fluoro-10-methyl-1,2-benzanthracenes is:

$$1{\cdot}3341\gamma < \Delta E_{1,\bar{3}} < 1{\cdot}4462\,\gamma$$

There are two exceptions only in the case of the methyl benzanthracenes to activity as defined by this criterion: both 5,8-dimethyl and 2'6-dimethyl 1,2-benzanthracene are allowed to be active whereas they are in fact inactive. The experimental data for the mono-fluoro derivatives are less complete; their most interesting feature is the dependence of the activity on fluoro-substitution in the '*K*' region, for the 4 isomer is strongly carcinogenic while the 3 derivative is inactive[5,6]. This is now consistent with theory which shows 3-fluoro-10-methyl-1,2-benzanthracene to fall well outside the active range with $\Delta E_{1,\bar{3}} = 1{\cdot}2824\,\gamma$. For those molecules which remain to be tested theory predicts:

(i) the 1'- and 2'-fluoro-10-methyl-1,2-benzanthracenes should be active; (ii) both the 5 and 8 isomers fall just a little outside our present 'active' range with $\Delta E_{1,\bar{3}}$'s of 1·3286 and 1·3327 respectively; since we have no molecules the activities of which are known with such low values of $\Delta E_{1,\bar{3}}$ we cannot be certain as to the probable activities of these compounds; and (iii) the 9-fluoro isomer has $\Delta E_{1,\bar{4}}$ lying within the active range. There seems no reason why the fourth unfilled level should not be used as the resonating charge transfer-level so that we would predict this molecule to be active. Evidence of inactivity would indicate that a common resonating level must be used by all the molecules of an isomeric series and would clearly be of considerable interest.

The interpretation of the activities of the methyl benzanthracenes has always been one of the most

Table 1. Differences in Energy between the Highest Filled and Successive Unfilled Levels in Units of γ, the Resonance Integral

Molecule	$\Delta E_{1,\bar{1}}$	$\Delta E_{1,\bar{2}}$	$\Delta E_{1,\bar{3}}$	$\Delta E_{1,\bar{4}}$	Activity
5,9,10 Trimethyl-	0·8711	1·1025	1·3868	1·5509	+
6,9,10 Trimethyl-	0·8733	1·1129	1·3919	1·5593	+
9,10 Dimethyl-	0·8769	1·1154	1·3978	1·5637	+
8,10 Dimethyl-	0·8842	1·1266	1·4122	1·5752	+
5,10 Dimethyl-	0·8848	1·1244	1·4114	1·5747	+
5,9 Dimethyl-	0·8851	1·1312	1·4156	1·5292	+
1'10 Dimethyl-	0·8909	1·1484	1·4229	1·5895	+
10 Methyl-	0·8909	1·1379	1·4229	1·5885	+
5,8 Dimethyl-	0·8867	1·1376	1·4252	1·5860	−
9 Methyl-	0·9931	1·1463	1·4287	1·5966	+
5,6 Dimethyl-	0·8910	1·1469	1·4309	1·5956	+
5 Methyl-	0·8065	1·1517	1·4387	1·6021	+
3 Methyl-	0·8977	1·1592	1·4390	1·6048	+
8 Methyl-	0·8974	1·1552	1·4409	1·6038	+
4 Methyl-	0·8975	1·1562	1·4411	1·6039	+
6,7 Dimethyl-	0·8984	1·1569	1·4413	1·6062	+
2'6 Dimethyl-	0·8985	1·1605	1·4428	1·6071	−
7 Methyl-	0·9002	1·1587	1·4462	1·6097	+
6 Methyl-	0·9014	1·1644	1·4462	1·6133	+
3'6 Dimethyl-	0·9004	1·1671	1·4473	1·6119	−
3'7 Dimethyl-	0·8996	1·1618	1·4477	1·6087	−
4' Methyl-	0·9022	1·1724	1·4480	1·6144	−
2' Methyl-	0·9012	1·1631	1·4485	1·6114	−
1' Methyl-	0·9046	1·1777	1·4523	1·6186	−
3' Methyl-	0·9038	1·1703	1·4536	1·6167	−
1' Fluoro-10-methyl-	0·8806	1·2190	1·4126	1·6184	?
2' Fluoro-	0·8274	1·0728	1·3794	1·5061	?
3' Fluoro-	0·8622	1·1402	1·4304	1·5736	+
4' Fluoro-	0·8173	1·1397	1·3379	1·5542	Toxic
3 Fluoro-	0·7911	1·0519	1·2824	1·4560	−
4 Fluoro-	0·8116	1·0381	1·3462	1·4609	+
5 Fluoro-	0·8161	1·0097	1·3286	1·4836	?
6 Fluoro-	0·8205	1·0771	1·3341	1·5773	+
7 Fluoro-	0·8321	1·0482	1·3667	1·5271	+
8 Fluoro-	0·8035	1·0233	1·3327	1·6199	?
9 Fluoro-	0·7806	1·0802	1·2712	1·4141	?

Note: For the methyl group, $\gg C_3—C_1 \equiv H_2$, the following parameters have been used:

$$\alpha_{C_3} = \alpha_C - 0{\cdot}1\beta$$
$$\alpha_{C_1} = \alpha_C$$
$$\alpha_{H_2} = \alpha_C - 0{\cdot}5\beta$$
$$\beta_{C_1 \equiv H_2} = 2{\cdot}5\beta_{C=C}$$
$$\beta_{C_1—C_3} = 0{\cdot}7\beta_{C=C}$$

For the fluorine atom:

$$\alpha_F = \alpha_C + 1{\cdot}0\beta$$
$$\beta_{C—F} = \beta_{C=C}$$

demanding requirements of any electronic theory of the mechanism of carcinogenesis. The success of the π complex model in this sense together with its explanation of the differences in activity between the 3- and 4-fluoro-10-methyl-1,2-benzanthracenes must be regarded as some further justification for its acceptance. On the other hand, the original difficulties of the model remain. The calculated energy-levels are based on an approximation which ignores the singlet-triplet separation of states arising through π electron excitation; moreover, this separation is not constant for all the carcinogenic alternant hydrocarbons although it is likely that it will be similar for the molecules discussed here. There is also the problem of the effects of mutual polarization on the energy-levels of the protein and hydrocarbon and its bearing on the likelihood, or otherwise, of the presence of resonating levels in the π complex. The attractiveness of the model undoubtedly lies in its simplicity and its ability to predict critical experiments. Davis *et al.*[7] have measured the enhanced semiconductivity of protein complexes and interpreted the results in a way consistent with the mechanism put forward here. Spectroscopical investigations of the hydrocarbon-protein complexes formed *in vivo* would clearly be also useful in defining the nature of the interaction. From the theoretical point of view, we hope to be able to examine the problem more realistically by the use of more sophisticated models for the carcinogens and protein since the present approach does not offer convincing evidence that the charge-transfer hypo-

thesis is a unique, rather than the most economical, description of the carcinogenic interaction.

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BIOCHEMISTRY

Hydrolysis of Hippuric Acid in Electrolytic Desalters

In 1947 Consden, Gordon and Martin[1] published the design of an apparatus for the removal of salts from solutions of amino-acids and peptides by electrolysis. Since that time this method has been frequently used for the preparation of samples for the analysis of urinary amino-acids by paper chromatography. Whenever this has been done little account seems to have been taken of the fact that hippuric acid, which is present in relatively large quantities in some urines, is a fairly strong organic acid, and for that reason approaches the semipermeable membrane of the desalter at an early stage in the electrolysis, and is there hydrolysed to benzoic acid and glycine. The purpose of this communication is to show that glycine released by the hydrolysis of hippuric acid is retained in the cathode compartment of the desalter, where it adds to the amount of free glycine already present in the urine, and that the increase in free glycine is dependent on the initial concentration of hippuric acid and salt.

In the course of work on the determination of amino-acids in urine using electrolytic desalting followed by paper chromatography[2–4], it was found that the mean 24-hr. excretion of glycine from ten subjects of European stock was 3·43 m.moles/24 hr., whereas the mean value obtained by Stein[5] using an ion exchange procedure was 1·76 m.moles/24 hr., and that obtained by Gutman and Alexander[6] using a chemical method was 1·60 m.moles/24 hr. The fact that some microbiological methods were giving values in the region of 10 m.moles/24 hr.[7], and that these high values were attributed to a greater activity of the micro-organism concerned for hippuric acid, led to the possibility that the high results obtained in my experiments could have been due to the retention of glycine in the cathode compartment subsequent to the hydrolysis of hippuric acid at the semipermeable membrane.

All the results quoted here were obtained with a small 'Perspex' electrolytic desalter using the same basic principle as that of Consden, Gordon and Martin[1], but designed for the rapid desalting of solution volumes not greater than 1 ml. In each case a starting current of 0·9 amp. and a voltage rising to about 200 V. was applied to the electrolysing solution. The fact that the hydrolysis of hippuric acid and the factors affecting it are not peculiar to a particular design of electrolytic desalter was shown when similar results were obtained from the electrolytic desalter of Astrup, Stage and Olsen[8].

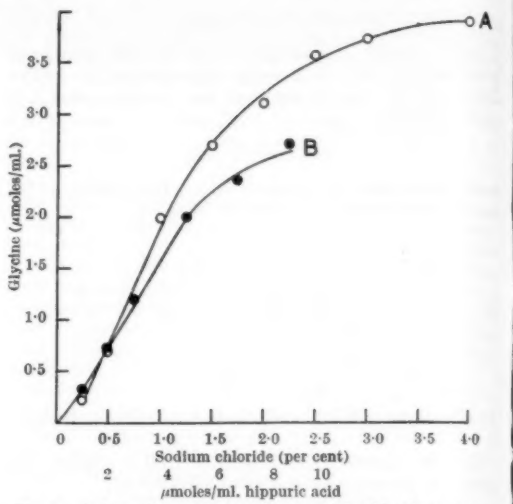

Fig. 1. *A* shows the amount of glycine retained in the cathode compartment when the initial concentration of sodium chloride is varied and the initial concentration of hippuric acid is constant at 5·0 μmoles/ml. *B* shows the amount of glycine retained in the cathode compartment when the initial concentration of hippuric acid is varied and the initial concentration of sodium chloride is constant at 1·0 per cent

The initial possibility that hippuric acid hydrolysis took place and resulted in the retention of free glycine in the cathode compartment of the desalter was examined by desalting a solution of hippuric acid in 1·2 per cent sodium chloride. A sample of the desalted solution was chromatographed and glycine was found to be present. A control was carried out by electrolysing a pure solution of hippuric acid for the same length of time, and this produced no free glycine. Desalting a solution of glycylalanine in 1·2 per cent sodium chloride produced no free glycine, which would seem to indicate that neutral peptides are unaffected.

A series of experiments was then carried out to determine the effect of changing concentrations of salt on the rate of hydrolysis of constant amounts of hippuric acid, and, also, the effect of changing amounts of hippuric acid in solutions in which the initial concentration of sodium chloride was kept constant. The results of these experiments are shown in Fig. 1. Each electrolysis was carried out by placing 1 ml. of the test solution in the desalter. No loss of glycine occurs during electrolysis in this desalter[9]. The desalting time varied from 2 to 15 min. depending on the initial concentration of sodium chloride.

Curve *A* shows that the amount of free glycine retained in the cathode compartment approaches an equilibrium between 3 and 4 per cent sodium chloride for a constant amount of hippuric acid. Curve *B* also shows a tendency to approach an equilibrium, and it is clear that the position of this equilibrium will be determined by the initial concentration of salt. Unfortunately, it was not possible to extend curve *B* any further due to an uncontrollable amount of heat being evolved at higher concentrations of hippuric acid.

All efforts to prevent the hydrolysis of the hippuric acid either by varying the starting current and voltage or the concentration of the sulphuric acid

flowing through the anode compartment resulted in failure.

No attempt has been made to examine experimentally the possible mechanisms involved in the retention of glycine in the cathode compartment, or of the hydrolysis of the hippuric acid at the semipermeable membrane. It seems reasonable to suggest, however, that the basic factor involved is the very low *p*H which, according to Consden, Gordon and Martin[1], prevails in the region of the semipermeable membrane. At the commencement of electrolysis the amount of chloride ion passing through the semipermeable membrane should be dependent on the initial concentration of sodium chloride. This movement of chloride ions from the cathode towards the membrane is presumably balanced by a similar movement of hydrogen ions supplied by the sulphuric acid on the anode side of the membrane. The concentration of hydrogen ions available in the region of the membrane would thus be determined by the amount of chloride ion approaching the membrane and would in turn determine the rate of hydrolysis of the hippuric acid together with the degree of retention of glycine in the cathode compartment.

These results show that great care must be taken when examining paper chromatograms of urinary amino-acids after electrolytic desalting, since the amount of free glycine found on the chromatogram will be seriously affected to a varying degree depending on the initial concentration of hippuric acid and salt in the urine. In general, electrolytic desalting should be avoided in any work involving the quantitative determination of glycine in urine.

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Purification of Fluorescent Protein Conjugates: Comparison of Charcoal and 'Sephadex'

Removal of unreacted fluorescent material from fluorescent protein conjugates is essential for unambiguous interpretation of microscopical findings in immunofluorescence[1]. Many procedures have been suggested for conjugate purification, but extraction with powdered activated charcoal[2] and gel filtration with 'Sephadex'[3,4] are at present the only efficient methods suitable for general application. These two methods have been compared as purification procedures for conjugates of lissamine rhodamine *B* (*RB* 200) and fluorescein isothiocyanate. We have confirmed the inefficiency of dialysis[5]: after exhaustive dialysis (7–10 days), conjugates still contain appreciable unreacted fluorescent material which can be removed by shaking with charcoal or passage through 'Sephadex'.

The powdered activated charcoal supplied by British Drug Houses, Ltd., for general decolorizing purposes has been found to be the most efficient material for removal of unreacted fluorescent material. It may contain soluble substances which affect the *p*H of the solution, but after thorough washing in phosphate-saline (0·15 *M* sodium chloride, 0·01 *M* phosphate; *p*H 7·1) and water, and drying at 100° C., it remains highly adsorbent. The effect of shaking different amounts of charcoal for 1 hr. with samples of the same conjugate has been examined by measuring the ratio of dye to protein concentrations. This ratio decreases to a constant value for both *RB* 200 and fluorescein isothiocyanate conjugates, as the amount of charcoal is increased (Table 1). For *RB* 200 conjugates, 15 mgm. charcoal per ml. of original serum is sufficient to reduce the ratio to its lowest value, but for fluorescein isothiocyanate conjugates, at least 75 mgm. per ml. is required; double these quantities are normally used to allow for small variations in conjugates. Only 5–10 per cent protein is lost from *RB* 200 conjugates, but the larger amount of charcoal for fluorescein isothiocyanate conjugates removes 30–40 per cent protein.

Table 1. Ratio of Dye to Protein in Conjugates extracted with Charcoal (μgm./mgm.)

Mgm. charcoal per ml. original serum	0	15	30	75	150	300
RB 200 conjugate	36	16	17	17	17	—
Fluorescein isothiocyanate conjugate	33	—	27	23	22	22

Purification by gel filtration is achieved by passing the centrifuged conjugate through a column of 'Sephadex *G*–25', which has been washed with phosphate-saline. The protein conjugate is eluted by phosphate-saline and travels more rapidly than the unreacted fluorescent material. A clear region develops between two coloured bands and the purified conjugate is collected first; there is very little loss of protein. The elution is continued to remove unreacted fluorescent material and clean the column for further use. Dilution of the conjugate (about three-fold) is of little consequence since the globulin fraction may be precipitated and reconstituted.

The antibody content of conjugated rabbit serum (anti-bovine-serum-albumin) has been measured by precipitation before and after purification by charcoal and by 'Sephadex'. The percentage recovery of antibody has been the same as the total yield of protein in each case, indicating that antibody is neither selectively removed from the conjugate nor denatured. *RB* 200 and fluorescein isothiocyanate conjugates of rabbit serum (anti-klebsiella) and goat serum (anti-rabbit-globulin), purified by each technique and brought to equal protein concentration, were examined for specific and non-specific staining and compared with dialysed samples. Both direct and sandwich staining were tested with a large series of histological and bacteriological preparations. No significant differences were found between charcoal- and 'Sephadex'-purified conjugates; both gave substantially less non-specific staining than the dialysed conjugates.

The convenience of a simple batch process recommends powdered activated charcoal as the most suitable routine purifying agent; it has always given uniformly satisfactory results with *RB* 200 conjugates. Our original experience with fluorescein conjugates was less satisfactory[5], but since using pure fluorescein isothiocyanate for conjugation and adopting the present standard procedure of charcoal extraction, consistently good results have been obtained. The 'Sephadex' method, although time-consuming and

less simple, is as efficient as charcoal extraction and may be preferred for fluorescein isothiocyanate conjugates of any weak sera which cannot be handled by sandwich staining.

We thank Dr. G. S. Warner for gifts of pure fluorescein isothiocyanate, and Prof. W. O. Kermack and Dr. M. G. McEntegart for helpful advice. The research programme has been supported by a grant from the Scottish Hospital Endowments Research Trust.

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Gas Chromatography of Androst-16-en-3α-ol and Related Steroids

During the course of work on the *in vitro* biosynthesis of androst-16-en-3α-ol (androstenol, I) from 2-^{14}C-acetate or ^{3}H-acetate by rabbit and guinea pig testes[1], it was found that the highly radioactive saponifiable lipids present could be only partially separated from the slightly active I on alumina, using as eluant light petroleum/benzene (1 : 1, v/v). Although in later experiments the neutral lipids were removed by saponification before column chromatography, it was considered that the technique of gas chromatography might provide an alternative method of separating (I) in view of recent results obtained in the steroid field[2–5].

We have used as stationary phase a non-polar silicone gum, *MS* 2211 (Midland Silicones, Ltd.). This was dissolved in toluene and stirred with sufficient acid-washed 'Celite 545' (85–100 or 100–120 mesh) to give 0·5, 1·5, 10 and 15 per cent (w/w) as already described[1]. Fig. 1 shows the separation of (I)[6] from a mixture of fatty acids up to C_{18}, using 15 per cent silicone gum on 'Celite 545'. With this relatively high concentration of stationary phase a fairly good separation was obtained but the retentions of columns having only 0·5 or 1·5 per cent were too small to allow adequate separation. Although such columns could well be of use in the analysis of bile acids (cf. VandenHeuvel, Sweeley and Horning[7]), they were not investigated further in relation to (I).

Using this 15 per cent column, an attempt has also been made to separate androstenol from a group of four steroids, similar to it both chemically and in their mobility on fully acetylated paper[1]. These compounds, all generously supplied by Dr. C. L. Hewett (Organon Laboratories, Ltd.), were the 3β-epimer of I (androst-16-en-3β-ol, II), ætiochol-16-en-3α-ol (III), androst-5: 16-dien-3β-ol (IV), and œstra-1 : 3 : 5 : 16-tetraen-3-ol (V). They were each applied to the column as 10 per cent (w/v) solutions in distilled methyl laurate, which contained enough myristic ester to serve as a reference substance. Analyses were carried out at six temperatures within the range 145–225°, using appropriate argon flow-rates and suitable chart-speeds in the recording potentiometer[8]. The results, together with the retention of each steroid relative to myristic ester and the calculated number of theoretical plates, are shown in Table 1.

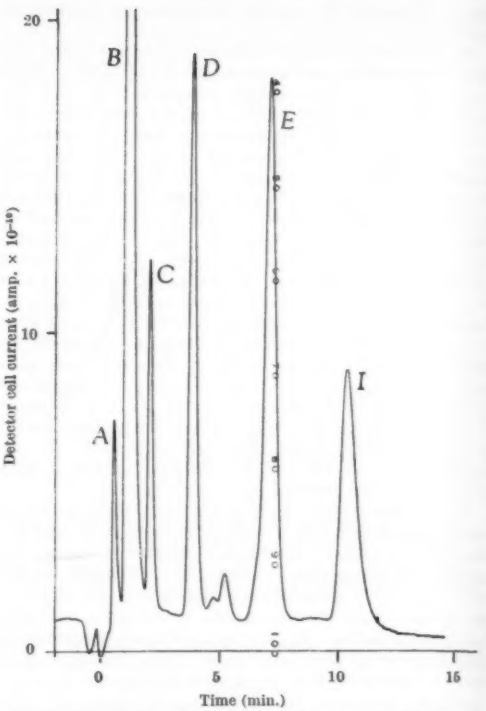

Fig. 1. Vapour phase chromatogram of 10 μgm. androsten-3α-ol (I), showing separation from methyl esters of (*A*) capric, (*B*) lauric, (*C*) myristic, (*D*) palmitic and (*E*) stearic acids. Stationary phase, 15 per cent silicone gum *MS*2211 on 'Celite', at 206° C.; argon flow-rate 35 ml./min.

Compounds III and V could be resolved from each other and from I, II and IV without difficulty, but the latter group of three steroids showed very similar properties and only partial resolution was achieved. Separation did not alter significantly over this range of temperature. Evidence of the thermal instability of I, II and IV was noticed at 185°, increasing markedly as the column was run at higher temperatures. Below 185°, however, the substances appeared stable even though they were retained for considerable periods.

After plotting the reciprocal of the absolute temperature against $\log_{10}$ (relative retention), it was clear that effective resolution of I, II and IV from one another would be possible with silicone gum as stationary phase in the range 150–180°, provided that columns equivalent to 45,000 theoretical plates were used. This should be possible with capillary

Table 1. Retentions relative to Methyl Myristate of some Steroids at Different Temperatures on a Silicone Gum Column

Temperature (° C.)		145	153	164	185	206	225
Steroids*	I	6·18	6.31	5·94	5·37	5·04	5·02
	II	6·45	6·45	6·05	5·43	5·11	5·00
	III	—	—	—	4·86	4·47	—
	IV	6·48	6·21	5·82	5·25	4·96	4·94
	V	—	—	—	6·81	5·96	—
Gas flow (ml./min.)		35	34	32	40	35	31
Theoretical plates (for methyl stearate)		4,210	1,950	2,240	1,460	1,200	930

* I, Androst-16-en-3α-ol; II, androst-16-en-3β-ol; III, ætiochol-16-en-3α-ol; IV, androst-5 : 16-diene-3β-ol; V, œstra-1 : 3 : 5 : 16-tetraen-3-ol.

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columns about 50 feet long[9]. The preparation of such a column is in hand.

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Effect of Insulin on Pathways of Glucose Oxidation in the Rat Lens

Experimental evidence as to whether lens is a tissue which responds to insulin has been inconclusive. Some investigators have found that glucose uptake by the isolated intact lens is increased after administration of insulin *in vivo*[1,2]. However, neither a direct effect *in vitro* on glucose uptake could be demonstrated[1,3], nor did insulin influence the accumulation of labelled glucose by the intact lens[4]. Recent work in this laboratory has shown that insulin causes a small but consistent increase of glucose uptake by the intact lens in Krebs–Ringer bicarbonate medium[5]. Kinoshita and Wachtl[6] have found that lens is capable of metabolizing glucose by both the Embden–Meyerhof pathway and the hexose monophosphate shunt. This communication deals with the effect of insulin on the two oxidative pathways in the rat lens and demonstrates an alteration of glucose oxidation in lenses of diabetic rats.

Lenses of rats of body-weight 100–105 gm. were excised as described in ref. 5. Diabetes was produced by intraperitoneal injection of 14·5 mgm. of alloxan/100 gm. body-weight; those animals having blood glucose-levels, when fed, of at least 350 mgm. per cent after three weeks were considered to be diabetic. Glucose-1-^{14}C and glucose-6-^{14}C were obtained from the Radiochemical Centre, Amersham, England. Carbon-14 labelled carbon dioxide produced during incubation was collected and counted as barium carbonate on a windowless gas-flow counter; corrections to infinite thinness were made according to an experimentally determined self-absorption curve. Results were calculated according to the following formula:

$$\mu\text{moles of added glucose converted to } {}^{14}CO_2 = \frac{\text{c.p.m. as barium carbonate}}{\text{specific activity of medium glucose}} \times 10^3$$

Values were multiplied by 10^3 in order to present the results as whole numbers since the absolute amount of conversion was low.

The stimulatory effect of insulin on the oxidation of glucose-1-^{14}C to carbon-14 labelled carbon dioxide may be seen in Table 1. This activation of the hexose monophosphate shunt by insulin was small (about 20 per cent) but significant ($P < 0.01$) and was consistently observed in all experiments. At the same time

Table 1. Effect of Insulin on the Oxidation of Glucose-1-^{14}C and Glucose-6-^{14}C to $^{14}CO_2$ in Rat Lenses

	Insulin	No. of exp.	μmoles of glucose converted to $^{14}CO_2 \times 10^{-3}$	Insulin effect (per cent)
Glucose-1-^{14}C	–	6	12·39 ± 0·43	
	+	6	15·01 ± 0·48	+ 21
Glucose-6-^{14}C	–	4	0·55 ± 0·04	
	+	4	0·29 ± 0·04	– 46

Incubation medium: 1·2 ml. of Krebs–Ringer bicarbonate buffer-*p*H 7·4, containing 5·8–6·5 μmoles non-labelled glucose plus labelled glucose to give specific activities of 147,000–176,000 c.p.m./μmole glucose. Insulin, when added, was 0·1 unit/ml. Incubated at 37° for 2 hr. in 95 per cent oxygen–5 per cent carbon dioxide. Each Warburg vessel contained three lenses (average weight, 25·2 mgm.) from rats fasted for 16 hr. Potassium hydroxide was injected through a special rubber stopper at the end of incubation, and after addition of sulphuric acid from side-arm the carbon dioxide released was collected for 45 min.

it may be seen that the oxidation of glucose-6-^{14}C to carbon-14 labelled carbon dioxide was depressed in the presence of insulin by about 50 per cent. This finding, although unexpected, has also been noted in experiments with mammary glands[7].

When these experiments were performed in a medium of Krebs–Ringer phosphate instead of bicarbonate, no insulin effect could be observed in either of the oxidative pathways.

Investigations on lenses from diabetic rats (Table 2) indicated that the production of carbon-14 labelled carbon dioxide from glucose was depressed in the diabetic state by about 20 per cent. The extent to which the oxidation of this sugar via the hexose monophosphate shunt was diminished in diabetes was of the same order of magnitude as the amount of activation observed when insulin was added *in vitro* to normal lenses. No consistent alteration in carbon-14 labelled carbon dioxide production from glucose-6-^{14}C could be demonstrated in lenses from diabetic rats.

Table 2. Oxidation of Glucose-1-^{14}C and Glucose-6-^{14}C by Lenses of Diabetic Rats

	Lenses used	No. of exp.	μmoles of glucose converted to $^{14}CO_2 \times 10^{-3}$	Percentage of change in diabetes
Glucose-1-^{14}C	Normal	4	13·82 ± 0·78	
	Diabetic	4	10·84 ± 0·09	– 22
Glucose-6-^{14}C	Normal	6	0·40 ± 0·04	
	Diabetic	4	0·37 ± 0·06	none

Incubation medium and conditions the same as in Table 1, except rats were fasted for two days.

The stimulatory effect of insulin on the hexose monophosphate shunt found in these experiments does not necessarily indicate that insulin is acting directly on the enzyme systems of this oxidative pathway. One of the present theories of insulin action is that it mediates in the transport of glucose into the cell. It is possible that insulin might, in this case, facilitate the transfer of glucose to that site in the lens where the hexose monophosphate shunt is operating.

Simultaneously with the increased activity of the direct oxidative pathway, a diminished production of carbon-14 labelled carbon dioxide from glucose-6-^{14}C takes place in the presence of insulin. Similar shifts, but in the opposite direction, have been reported by Lerman[8,9], who found that the hexose monophosphate shunt was depressed in galactose-fed and in old rats while production of carbon-14 labelled carbon dioxide via the Embden–Meyerhof and Krebs cycle was increased. Our experiments have shown that, in diabetes also, the hexose monophosphate shunt is depressed; the corresponding increase in carbon-14 labelled carbon dioxide production from glucose-6-^{14}C was, however, not observed, possibly because the absolute amount of radioactivity resulting from this pathway was so low that small differences

could not be detected. It would appear that at least one common denominator exists in all three of these cataractogenic conditions, namely, a diminished activity of the hexose monophosphate shunt.

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Isolation of an Unknown Auxin from a Solution of Indole-3-Acetic Acid labelled with Carbon-14

It is desirable in certain investigations in metabolism to work with as stable a compound as possible, or use as an indicator of the basic structure of the compound some form of marker or tag. Such is the case with plant growth regulators and auxins.

In the case of indole-3-acetic acid (IAA) it is known that the carboxyl group is quite reactive and even the methylene group may be degraded. Thus, there is advantage in labelling an atom in one of the rings. This has been done by Pichat *et al.*[1] at position 2 in the indole ring.

This material was obtained for work on translocation of IAA in the *Avena* coleoptile. Approximately four months after its synthesis very little activity could be found in the labelled material. The substance was known to be unstable from radiolysis (internal irradiation from the isotope) by the carbon-14 on some part of the molecule.

The solution contained 2·5 mgm. of IAA in methanol. Its specific activity was originally 6·9 mc./mm. No suggestion has been made as to the degradation product(s). This may prove to be one of those recently suggested by Fawcett[2].

In order to determine the purity of the solution and to find out where the radioactivity was located, that is, if in the IAA or some breakdown product, a chromatographic separation of the methanol solution was made.

This had been reported (private communication) by K. V. Thimann in June 1960. He had made a chromatographic analysis of the solution and found that most of the radioactivity was in a spot near the solvent front and very little in the IAA at 0·30. The method of Stowe and Thimann[3] was used with isopropanol/ammonia/water (10:1:1) to identify the auxin. The chromatogram for the IAA solution gave two spots when sprayed with Salkowski reagent[4], one at about R_F 0·33 (for IAA) and another (salmon-orange colour) at about R_F 0·90.

The strip was placed under a Geiger counter and read for radioactivity. All of the counts were at R_F 0·90 and none at R_F 0·33. This corroborated the results of Thimann.

A test was made to determine which compound was an active auxin. The spots (before development with Salkowski) were cut from the paper strip, placed in cold peroxide-free ether in flasks in the refrigerator overnight. The flasks (30 ml.) were placed on a shaker for a short time, then the ether was evaporated down before being applied to 1·5 per cent agar medium.

A Went *Avena* bioassay was run on these materials. A 50 μgm./l. sample was run as a control. This gave a normal curvature of about 13°.

The ether extracts from the IAA spot produced curvature. However, when the eluted spot was sprayed with Salkowski after extraction, an easily detectable spot was developed. This indicated that much of the IAA was not eluted from the spot by ether.

When the same test was made on the *X* substance, curvature resulted. On spraying with Salkowski reagent, no colour developed at the spot for the *X* compound.

One further test was conducted to see if possibly the IAA was being carried along to the *X* spot and resulting in a tail between the two spots. There was no radioactivity in any area other than at the *X* spot of R_F about 0·90.

Strips were cut from the paper between the IAA spot and *X* spot and eluted with ether. No curvature resulted from these areas, whereas it consistently occurred for the *X* compound.

Because of the lack of more compound (original sample only 2·5 mgm.) only three runs were made on the *X* compound and the blank areas between the two spots.

An attempt was made to obtain an ultra-violet spectrum for the substance, but due to interfering (brown) degradation colours of indole derivatives, no satisfactory spectrum could be obtained.

Although closer to the chemical structure of IAA, the new auxin described by Post[6], skatylglyoxal hydrate, does not appear to coincide in both requirements. Its *Avena* curvature seems similar, especially in being more active than IAA, but the chromatographic analysis does not behave in the same pattern, although in no case was the solvent system exactly the same.

An unknown, *X* compound was shown to contain all the radioactivity from an initial preparation of β-(¹⁴C-2-indole) acetic acid. This compound also possessed auxin properties as shown by its *Avena* coleoptile curvature ability. Further investigations will be attempted when more of the labelled IAA is available and more quantitative runs can be made.

Other compounds were run in both the bioassay and chromatographic analysis, but none possessed the characteristics of the *X* compound. Such compounds as indole, skatole, mixtures of IAA and the former two, other indole compounds, and the two non-indolic auxins of Crosby and Vlitos[5], 1-docosanol and 1-octadecanol, were run. None was found to fit the dual patterns of the *X* compound; none showed *Avena* curvature. It does not seem likely that the two compounds of Crosby and Vlitos could be formed from

the original compounds or the degradation products in the Pichat synthesis[1].

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Distribution of Trypsin Inhibitors in the Sera of Various Animals

Trypsin inhibitors have been found in pancreas, serum, colostrum, egg-white, soya bean, and lima bean[1]. They have all been crystallized except from egg-white[2]. Thus, examination of the distribution of trypsin inhibitors in proteins of them appears to be out of the question. Recently, Nakamura *et al.*[3] have demonstrated, by two-dimensional paper electrophoresis, that two trypsin inhibitors were contained in the extract of soya-bean. Hence the distribution of trypsin inhibitors in other biological fluids deserves to be re-investigated to see whether there are also two inhibitors in them or not.

In Fig. 1 is shown one of the 'crossing diagrams' of human serum against trypsin. The electropherogram shown was obtained by two-dimensional electrophoresis as follows : Human serum was applied on line *AB* and the first electrophoresis was carried out in direction 1. A solution of trypsin was then applied on line *XY* and the second electrophoresis was carried out in direction 2. Under the given experimental conditions, trypsin migrates towards the cathode and serum proteins towards the anode. Thus the former migrates over the lines of the latter. As can be seen from Fig. 1, the lines of serum proteins were partially digested by trypsin. In this way the front of the trypsin line, which is named the 'crossing diagram' of the serum against trypsin, appeared across the zones of serum proteins. It shows two grooves (or peaks, if viewed conversely), one in the zone of α_1-globulin and the other in that of α_2-globulin. At the grooves in the line, trypsin is retarded. The retardation of the migration of trypsin must have been caused by the reaction with substances in serum, which combined with it and inhibited its activity. Therefore from the 'crossing diagram' of serum proteins against trypsin the distribution of trypsin inhibitors in serum can be investigated easily. Fig. 1 shows that human serum contains two trypsin inhibitors, one in α_1- and the other in α_2-globulin.

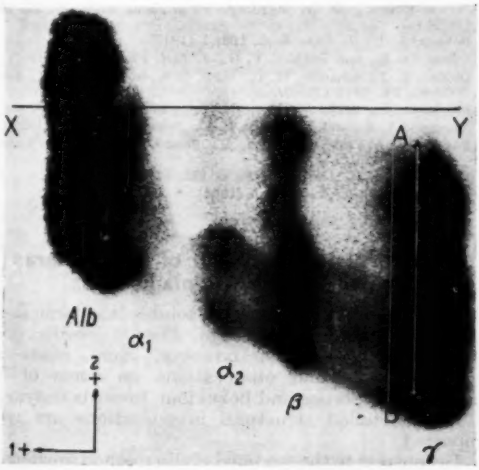

Fig. 1. 'Crossing diagram' of human serum against trypsin. First electrophoresis, 0·04 ml./6 cm. of human serum were applied on line *AB* and run at 100 V. for 16 hr. Second electrophoresis, after the first one, 0·1 ml./14 cm. of 1·5 per cent crystallized trypsin solution was applied on line *XY*, and run at 100 V. for 8 hr. Barbiturate buffer, *p*H 8·6 and ionic strength 0·05. Stained with bromophenol blue

Table 1 shows some of the results obtained by the same method with sera of various animals. From the results obtained so far, it can be inferred that sera of many animals contain two trypsin inhibitors (although some contain only one) and the distribution of them in the serum proteins is characteristic of the species. The circumstances are the same as for the 'crossing diagrams' of serum against protein *J* of jack beans[4].

Table 1. Distribution of Trypsin Inhibitors in Sera of various Animals

Animal	Albumin	α		$\beta\,\gamma$
Human		+++	+	
Dog				++
Cat		++		++
Horse	++		++	
Cattle			+++	
Sheep			+++	+
Pig			+++	
Rabbit		+++		+
Guinea pig		+++		+

The number of (+) denotes rough estimation.

The method of two-dimensional electrophoresis to obtain the 'crossing diagram' is not strictly quantitative, but from the height of the peak, if other conditions were the same, a rough comparison can be made between the sera of different animals. In human sera particularly, the changes of distribution of trypsin inhibitors in diseases deserve further investigation.

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Trehalose in the Cellular Slime Mould *Dictyostelium mucoroides*

In 1925 Iwanoff[1] demonstrated the presence of the non-reducing disaccharide, trehalose, in isolated spores of the acellular slime mould, *Reticularia lycoperdon* (Myxomycetes). Recent work[2] on soluble carbohydrates in the cellular slime moulds have not mentioned the presence or absence of this sugar. The present report concerns the occurrence of

trehalose in the cellular slime mould *Dictyostelium mucoroides* (Acrasiales), and preliminary work on the role of this sugar during development.

The fruiting body of *D. mucoroides* consists of a long cellular stalk that bears a spherical mass of spores. On germination each spore releases a small 'amœba'. These amœbæ feed on bacteria, grow, and divide. When the bacteria have been consumed, aggregation of the amœbæ occurs at various locations on the culture dish forming cigar-shaped masses of cells, pseudoplasmodia. The pseudoplasmodia migrate and each constructs a stalk as it does so. Eventually migration ceases and those cells that have not become stalk cells are transformed into spores. In certain strains of this species post-aggregative development results in the formation of a multi-cellular encystment stage, the macrocyst, instead of a fruiting body. The present work is concerned mainly with spores and macrocysts, but vegetative amœbæ and pseudoplasmodia were also investigated.

Spores were plated together with *Escherichia coli* on the nutrient agar medium of Bonner[3]. When fruiting occurred, the spores were harvested, washed, and homogenized in a glass homogenizer with 80 per cent methanol. Macrocysts were collected by plating spores of the proper strain on the same medium and flooding the culture with about 3 ml. of sterile distilled water. Under these conditions very few fruiting bodies and large numbers of macrocysts were formed. The latter were collected, washed, and homogenized in 80 per cent methanol. Following centrifugation the alcoholic extract was evaporated to dryness and redissolved in distilled water. This solution was chromatographed on Whatman No. 1 chromatographic paper for the separation, elution, and quantitative determination of carbohydrates[4]. The solvent systems used were *n*-butanol/95 per cent ethanol/acetone/water (5 : 4 : 3 : 2), *n*-propanol/ethyl acetate/water (7 : 1 : 2), ethyl acetate/pyridine/water (8 : 2 : 1), and *n*-butanol/acetic acid/water (4 : 1 : 5).

When extracts of various stages of the life-cycle were analysed by paper chromatography, large amounts of an alcohol-soluble carbohydrate were detected in the spores and macrocysts but not in the other stages. This substance migrated with known disaccharides and was shown to be trehalose by the following criteria: it was non-reducing unless oxidized first with periodate[4]. After elution from the chromatogram it reacted with the anthrone reagent[5] and on hydrolysis with 1 *N* hydrochloric acid liberated only glucose as judged by use of the glucose oxidase system. ('Glucostat', obtained from the Worthington Biochemical Corp., Freehold, New Jersey, was used for the assay of glucose.) When an eluted sample was co-chromatographed with authentic trehalose, no separation was observed in the four solvent systems listed above. Treatment of an eluted sample with a partially purified preparation[6] of the specific hydrolytic enzyme, trehalase, readily hydrolysed the sample exclusively to glucose.

Quantitative work carried out on isolated spores showed that trehalose constituted more than 7 per cent of the dry weight, and a similar concentration was found for the macrocysts. Trehalose could not be detected in the other life-cycle stages (limits of detection < 0·1 per cent of the dry weight) but small amounts of glucose were always present as judged by paper chromatography. Fructose has been reported present in *D. discoideum*[2], but we have not detected this sugar in our material. It appears that trehalose and small amounts of glucose are the main, and perhaps the only, soluble carbohydrates present in *D. mucoroides*.

The restriction of trehalose to the spores and macrocysts implied that this sugar was utilized during germination. Accordingly, we incubated spores for 12 hr. under conditions conducive to germination, and then analysed the resulting amœbæ for trehalose content. No trehalose was detected. Although a more complete balance sheet is necessary before any conclusions can be drawn, it appears that trehalose might play some part in the germination process of this organism, possibly as an energy source for this activity.

Trehalose has been found in large amounts in the resistant or dormant stages of several other organisms such as the ascospores of *Neurospora*[7], the eggs of *Ascaris*[8] and *Bombyx mori*[9], and the resistant embryo of *Artemia*[10]. In addition, we have found large amounts of trehalose in the desiccatable eggs of *Aedes aegypti* and dried statoblasts of the bryozoan *Plumatella* (unpublished observations). Moreover, in the majority of these cases the amount of trehalose decreases sharply during the resumption of 'activity', again indicating a role for this sugar during the transition from the dormant to the active state. We are at present extending our observations to resistant stages of representatives from other phyla.

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Structural Investigation of a Laminaran isolated from *Eisenia bicyclis*

In 1939 Nisizawa[1] isolated a soluble laminaran from the fronds of the brown alga, *Eisenia bicyclis*. No further investigations, however, were made at that time, beyond observations on some of its chemical properties and behaviour towards enzymes. Recent detailed structural investigations are now reported.

In contrast to the modified Kylin method previously used by Nisizawa[1], the small pieces of frond were extracted with about ten times their volume of 1 per cent hydrochloric acid at room temperature for five days. The crude laminaran was precipitated from the extract by the addition of five volumes of

Table 1

Oligosaccharide fraction	*F*1	*F*2	*F*3	*F*4	*F*5	*F*6	*F*7	*F*8	*F*9	*F*10
Yield (gm.)	12	11·5	1·6	0·9	2·2	0·8	0·5	0·4	0·4	0·1
$[\alpha]_D^{20}$	+9·6	+20·4	—	−1·5	+2·4	−2·4	—	—	—	—
D.P.*	2·0	2·0	3·1	3·0	3·1	3·1	3·9	3·9	3·9	4·0
Disaccharide produced from each fraction†										
Laminaribiose	−	+	−	+	+	+	−	+	+	+
Gentiobiose	+	−	+	+	−	+	+	+	+	−

* Ratio of the reducing powers before and after complete hydrolysis.

† Identified by paper chromatography.

methanol. Purification was effected by repeated treatment with charcoal and precipitation with methanol from aqueous solutions.

The final product, obtained as a white powder after electrodialysis, was very soluble in water and nitrogen-free (Kjeldahl). Ash (direct), 0·1 per cent. Found, C, 44·44 per cent, H, 6·22 per cent, $(C_6H_{10}O_5)_n$; requires, C, 44·44 per cent, H, 6·17 per cent.

Eisenia laminaran reduced hypoiodite equivalent to an average chain-length of 18. This is close to the value, 21, obtained by cryoscopy[1] and thus almost all molecules of this laminaran, in contrast to the insoluble[2,4,5] and soluble[3] laminarans from the genus *Laminaria*, appear to possess a reducing group. No mannitol was detected by paper chromatography of the laminaran hydrolysate after removing glucose by alkaline degradation and by fermentation.

The specific rotation of the laminaran was the same as that reported by Nisizawa[1], $[\alpha]_D^{20}$, − 45·5° (in water), although the extraction method was essentially different. The acetyl derivative had $[\alpha]_D^{20}$, − 42·2° (in chloroform). Intrinsic viscosities were 1·35 (in water) for free laminaran, and 3·87 (in *n*-cresol) and 5·9 (in chloroform) for laminaran acetate.

The laminaran consumed 0·73 mole periodate and released 0·37 mole formic acid per mole anhydroglucose when oxidized by 0·03 *M* sodium metaperiodate at room temperature for 24 hr. These values were much higher than those for insoluble laminaran[3,4].

To obtain partial hydrolysis products, laminaran (150 gm.) was acetolysed at 35° C. for 96 hr. and the mixture of deacetylated saccharides (121 gm.) was fractionated by charcoal-'Celite' column chromatography. Ten different oligosaccharides were isolated. The yield $[\alpha]_D$, D.P. and the identity of disaccharides produced from the oligosaccharides on partial acid hydrolysis are shown in Table 1. Of the ten oligosaccharides obtained, two were disaccharides, four were trisaccharides and another four were tetrasaccharides. All these appeared to contain only β-1 : 3- and/or β-1 : 6-links. Table 2 shows the periodate oxidation data for these oligosaccharides and their possible structures. Maximum periodate consumption and formic acid release increased by 2 moles and 1 mole, respectively, for some oligosaccharides when 0·06 *M* periodate was used in place of 0·03 *M*, whereas the values for the others were the same at both periodate concentrations. The former group may be terminated by a reducing glucose residue which was subject to over-oxidation, possibly joined by a β-1 : 3-link, whereas the latter group may be terminated by a reducing residue which was resistant to over-oxidation, possibly being joined by a β-1 : 6-link. From the maximum values obtained on periodate oxidation and the results in Table 1, the fractions from *F*1 to *F*6 were identified as gentiobiose, laminaribiose, gentiotriose and 6-β-laminaribiosylglucose, laminaritriose and 3-β-gentiobiosylglucose, respectively.

Although 3 : 6-di-*O*-β-glucosylglucose would be expected to produce the same maximum values on periodate oxidation as laminaritriose when 0·06 *M* periodate was used, they may be differentiated on the basis of their maximum values in 0·03 *M* periodate since only laminaritriose would be expected to show a lowered value. Furthermore, *F*5 did not give gentiobiose on partial acid hydrolysis and gave glucose after being oxidized with periodate and hydrolysed. This behaviour indicates the absence of 3 : 6-di-glucosylglucose. The absence of this trisaccharide suggests in all probability a linear structure for *Eisenia* laminaran. Results with methylation also support this assumption. Although three different methylglucoses were obtained, one of which was identified as 2 : 3 : 4 : 6-tetramethylglucose and the other two as 2 : 3 : 4- and 2 : 4 : 6-trimethylglucoses on the basis of their methoxyl contents and R_F-values, no dimethyl glucoses were found on the paper chromatogram. Of the four different tetrasaccharides, *F*8 and *F*9 could not be identified, while the characteristic patterns of maximum values for *F*7 and *F*10 suggest that the latter two may be gentiotetraose and laminaritetraose, respectively. It should be noted that gentiotriose and gentiotetraose have never been found in the partial acid hydrolysates of *Laminaria* laminarans[2,3].

The alkaline degradation of *Eisenia* laminaran in 4 *N* as well as 2 *N* sodium hydroxide solutions at 30° C. ceased after the loss of about one-third of the weight of the laminaran, whereas periodate uptake and formic acid release by the residual polysaccharide was nearly 1·4 times that of untreated laminaran. The part of the laminaran removed appears, therefore, to consist of β-1 : 3-linked glucose residues which would not contribute to the periodate consumption. The cessation of alkaline degradation may reflect the presence of a β-1 : 6-linkage in the molecule, as discussed in ref. 6. Thus it may be suggested that *Eisenia* laminaran is a linear β-glucan with a D.P. of about 20 containing both 1,3 and 1,6 linkages (in the approximate proportion 2 : 1), and since gentiotetraose was isolated from this laminaran by partial acid hydrolysis, at least three β-1 : 6-linked glucose residues must be present as a block among the β-1 : 3-linked residues, probably occupying about the central region of the molecule.

Table 2

Oligosaccharide fraction	Maximum value observed (moles) Periodate	Formic acid	Possible structure	Maximum theoretical value (moles) Periodate	Formic acid
*F*1	4·9 (4·8)	3·9 (3·8)	G1–6 (G)	5 (5)	4 (4)
*F*2	5·3 (3·2)	2·7 (1·9)	G1–3 (G)	5 (3)	3 (2)
*F*3	7·1 (6·8)	4·9 (—)	G1–6G1–6 (G)	7 (7)	5 (5)
*F*4	5·2 (4·9)	3·8 (3·7)	G1–3G1–6 (G)	5 (5)	4 (4)
*F*5	4·7 (2·6)	2·9 (1·8)	G1–3G1–3 (G)	5 (3)	3 (2)
*F*6	7·2 (4·8)	4·0 (3·3)	G1–6G1–3 (G)	7 (5)	4 (3)
*F*7	8·9 (8·7)	5·8 (6·2)	G1–6G1–6G1–6 (G)	9 (9)	6 (6)
*F*8	6·8 (6·9)	4·9 (—)	G1–6G1–3G1–6 (G) G1–3G1–6G1–6 (G) G1–3G1—6 (G) 6–1G	7 (7)	5 (5)
*F*9	7·0 (4·9)	4·0 (2·7)	G1–3G1–6G1–3 (G) G1–6G1–3G1–3 (G) G1–3G1–3 (G) 6–1G	7 (5)	4 (3)
*F*10	5·2 (2·8)	2·0 (2·7)	G1–3G1–3G1–3 (G)	5 (3)	4 (3)

Note: G, β-linked glucose residue; (G), glucose residue with reducing group. For the figures without parentheses, 0·06 *M* sodium metaperiodate, at room temperature, for 144 hr. For the figures with parentheses, 0·03 *M* sodium metaperiodate, at room temperature, for 24 hr.

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Occurrence of Sedoheptulose in Carpophores and Basidiospores of *Inocybe* Species

Although basidiospores of various xylophilous, coprophilous and lignicolous Hymenomycetes germinate quite readily, and in high percentage, those from mushrooms with proved mycorrhizal associations rarely, if ever, respond to standard mycological techniques. Among the latter are species of *Amanita*, *Lactarius*, *Cortinarius*, *Russula*, *Hygrophorus*, and *Boletus*. After completing an extensive series of experiments on the above problem, Kneebone[1] concluded that the low respiratory-rate of the spores is probably not limited by a lack of metabolic reserves but rather by some deficiency of the organism which prevents efficient utilization of available respiratory substrates.

We are investigating physiologically active constituents in the carpophores of various species of *Panaeolus* and *Inocybe* native to the Pacific northwest of the United States. *Inocybe* species are thought to be mycorrhizally associated and have proved extremely difficult to sub-culture either from carpophore tissues or spores. Information on the nature of the carbohydrate reserves of carpophores and spores was needed.

In a paper chromatographic study of extracts of carpophores of 91 species representing 29 genera of fleshy fungi, Bonnet[2] found mannitol in 86, trehalose in 82, and glucose in 34. The results of Sussman and Lingappa[3] suggested that trehalose is the probable substrate the utilization of which is correlated with the germination of ascospores of *Neurospora tetrasperma*. However, Kneebone had reported that the stimulatory effects of heat shock and of furfural on the germination of *Neurospora* ascospores could not be duplicated on spores of the mycorrhizal-associated fungi which he tested.

Dried carpophores of several species of *Inocybe*, ground to a No. 40 powder, were each shaken for 18–24 hr. at room temperature with 20 ml. of 70 per cent ethanol per gram of powder. The marcs were discarded and the clear filtrates extracted with petroleum ether to reduce the fat and oil contents.

Following evaporation *in vacuo* at 38° C., the residues were dissolved in 1·5 ml. of 50 per cent isopropanol, transferred to vials and chilled overnight to remove interfering salts[4]. Portions (25–50 μl.) were spotted on sheets of Whatman No. 1 filter paper and irrigated by the circular method of Rutter[5] in 150-mm. Petri dishes with *n*-butanol/pyridine/water (6:4:3) or *n*-butanol/ethanol/water (10:1:2), for 2 and 7 hr. respectively. When processed with the ammoniacal silver nitrate spray of Partridge[6] or the periodate–permanganate spray of Lemieux and Bauer[7], three to five distinct zones appeared. One of these was identified as mannitol by comparison of its R_F value with that of reference mannitol.

Volemitol (D-*manno*-D-*talo*-heptitol[8]) was isolated many years ago by Bourquelot[9] from carpophores of *Lactarius volemus*. The possibility that seven-carbon saccharides might also occur in *Inocybe* species, plus the apparent specificity of colour reactions for ketoses reported by Benvenue and Williams[10], prompted us to utilize a spray containing orcinol. After irrigation in the solvent systems previously mentioned, the papers were sprayed with water-saturated *n*-butanol–orcinol–trichloracetic acid[11] and heated for 10 min. at 105° C. Blue bands with low R_F values were noted from carpophore extracts of the following species of *Inocybe*: *picrosma* Stuntz, *kauffmanii* A. H. Smith, *cinnamomea* A. H. Smith, *lilacina* (Boud.) Kauff., *mixtilis* (Britz.) Sacc. sensu Kühner, *subdestricta* Kauff., *geophylla* (Fr.) Quél. var. *lateritia* Weinm., *agglutinata* Peck, and *lacera* (Fr.) Quél. *I. albodisca* Peck, *I. sororia* Kauff., and *I. xanthomelas* Boursier and Kühner in Kühner did not show detectable amounts.

The concentrations of the ketoheptuloses in the extracts were very low. *I. kauffmanii* gave the strongest tests but our supply of dried carpophores was limited. The mobilities of ketoheptulose from *I. cinnamomea* and *I. kauffmanii* extracts, which had been partially purified by a series of treatments including defatting, deproteinization, and ion-exchange, were compared with those of crystalline standards of L-*gala*-heptulose, D-*gluco*-heptulose, D-*manno*-heptulose, and D-*allo*-heptulose. Partially purified sedoheptuloses from *Cotyledon glauca* Baker and *Sedum spectabile* Bor. were also included.

A 48-hr descending irrigation technique, which allowed the solvent mixture (*n*-butanol–pyridine–water) to drip off the paper, was necessary for clear-cut separation. Although comparative R_F values could not be obtained, the mobilities of heptulose from the two species of *Inocybe* were identical with reference sedoheptulose, separately and in admixture.

The smooth-walled spores of *I. cinnamomea* were subjected to low-temperature cavitation in a Raytheon oscillator (10 kc./s.). About 60 min. were required to disrupt approximately 90 per cent of 100 mgm. of spores in 20 ml. of water together with some 0·2 mm. diameter glass paving beads. The mobility of spore heptulose in the descending system was the same as that obtained from the sporocarps of *I. cinnamomea*. A second ketose was also present in the partially purified spore extract. It gave a bright yellow colour with the spray containing orcinol and had a mobility similar to that of fructose. Angular spores from *I. mixtilis* and *I. xanthomelas* were far more refractory to treatment and similar amounts did not give positive heptulose tests. Much greater quantities of spores would be required to obtain enough heptulose for positive chemical identification. This would indeed be difficult considering that approximately 6,000 caps of *I. cinnamomea*, averaging about 1 in. in diameter, yielded only 800 mgm. of spores.

This constitutes the first report of the occurrence of sedoheptulose in extracts of mushroom tissues.

The possibility that one of the oxidative pathways in these spores may proceed through the pentose–phosphate cycle remains to be proved.

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Inhibition of Dopamine β Oxidase by Adrenalone

WE have reported that several structural analogues of dopamine are inhibitors of dopamine β oxidase. Dopamine β oxidase catalyses the conversion of dopamine to norepinephrine and the enzyme activity was determined by fluorimetric estimation of norepinephrine formed, by the method of von Euler *et al.*[1]. However, when adrenalone was tested as a possible inhibitor of dopamine β oxidase, the fluorimetric assay of norepinephrine was unsatisfactory. This was due to the fluorescence quenching of norepinephrine by adrenalone. The fluorescence quenching by other keto compounds has been reported[2], and such quenching may represent a serious obstacle to the fluorimetric determination of catecholamines in the presence of ketones such as adrenalone.

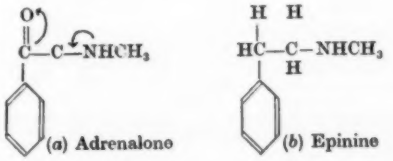
Therefore, in these investigations, where adrenalone was tested as a possible inhibitor of dopamine β oxidase, dopamine-^{14}C was used as a substrate. The amount of dopamine-^{14}C which disappeared from the incubation mixture as well as the norepinephrine-^{14}C formed in the incubation mixture were calculated from the radioactivity of each of these compounds. The incubation procedure as well as the separation of dopamine-^{14}C from norepinephrine-^{14}C was carried out as already described by us (ref. 3). The degree of the rate of inhibition of dopamine β oxidase was determined by comparing the amount of norepinephrine-^{14}C formed and the amount of dopamine-^{14}C which disappeared in an incubation mixture which contained only the substrate and in an incubation mixture which contained adrenalone and the substrate.

Table 1 shows the rate of inhibition of adrenalone on dopamine to norepinephrine conversion. For comparison, the rate of inhibition of dopamine to norepinephrine by epinine is also presented in Table 1. It is evident that adrenalone is a more potent inhibitor of the dopamine to norepinephrine conversion than epinine.

Table 1. INHIBITION OF DOPAMINE TO NOREPINEPHRINE CONVERSION BY ADRENALONE

Amount added (μmoles)			Norepinephrine formed	Dopamine disappearing
1·5	none	none	0·60 ± 0·06	0·80 ± 0·08
1·5	none	12	0·45 ± 0·05	not estimated
1·5	12	none	0·20 ± 0·02	1·2 ± 0·1

The structure of adrenalone (*a*) differs from epinine (*b*) only in that the former possesses a ketone group in the β position.



(*a*) Adrenalone (*b*) Epinine

The electron-withdrawing effect of the carbonyl group in adrenalone will tend to make the nitrogen of the secondary amino group less basic. This is consistent with our previous findings that primary amines (which are weaker bases) are also stronger inhibitors of dopamine β oxidase[3]. A second possibility is that the electrophilic centre of adrenalone interacts with the nucleophilic centre on the enzyme surface.

It should be stressed that while epinine itself is also a substrate of dopamine β oxidase and its inhibition of dopamine to norepinephrine conversion is due to a substrate competition, it is more likely that adrenalone is a non-competitive inhibitor.

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Acidic Dissociation of 7 : 9-Dialkylguanines and its Possible Relation to Mutagenic Properties of Alkylating Agents

Watson and Crick[1] proposed as a possible mechanism for mutation that changes in the tautomeric structure of a base of deoxyribonucleic acid (DNA) could induce its pairing with an anomalous base. In considering the possible correlation between the action of alkylating agents on DNA and the mutagenic activity of these agents, we mentioned in a recent report[2] that it was not obvious how the initial action of alkylation of DNA, which has been shown to occur at $N_{(7)}$ of guanine moieties[3,4], could affect base-pairing in DNA according to the Watson–Crick model. We therefore suggested that the subsequent loss of the alkylated guanine moiety from DNA (refs. 4 and 5) might be the chemical action necessary for mutation to result.

We now wish to present preliminary evidence that alkylation of guanine nucleosides or other 9-substituted guanines at $N_{(7)}$ results in an immediate change in the molecular structure of these bases at neutral *p*H in that part of the molecule involved in hydrogen-

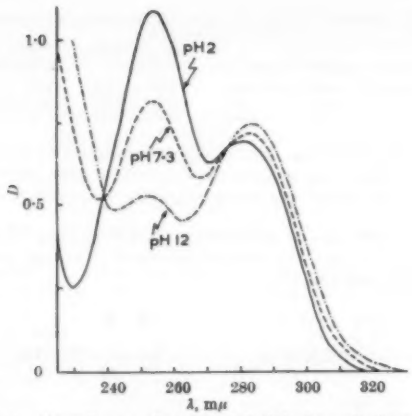
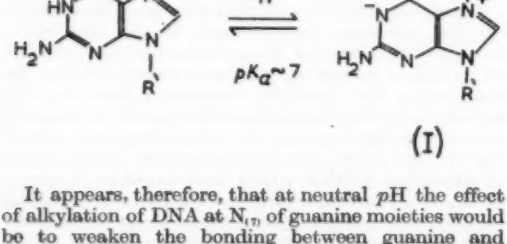


Fig. 1. Absorption spectra of 7 : 9-di-(2′-hydroxyethyl)guanine (0·0267 mgm./ml.) at *p*H 2, *p*H 7·3 and *p*H 12, 20°

bonding as required by the Watson–Crick model. This follows from investigation of the acidic dissociation of 7 : 9-disubstituted guanines by spectrophotometric titration. The absence of a basic *pKa* in the region of 3 for 7 : 9-dialkylguanines has previously been noted[6]. However, with increasing *p*H in the region of *p*H 7 the absorption spectrum of 7 : 9-dialkylguanines changes, the change being complete at about *p*H 9, and these changes are consistent with the occurrence of an acidic dissociation in the region of *p*H 7, that is, about two units of *p*H lower than for the corresponding 7 or 9-monosubstituted guanines. Fig. 1 shows spectra for 7 : 9-di-(2′-hydroxyethyl)guanine and Fig. 2 for 7-methylguanosine.

The spectrum of the molecular species of 7 : 9-disubstituted guanines with zero net charge, that is, at *p*H greater than 9, resembles that of 7- or 9-monosubstituted guanines in alkali. This suggests that the proton has been lost from the $N_{(1)}$ ring-nitrogen atom. Conventional formulation of these bases must therefore be as a zwitterion, as represented in (I), or with the negative charge on the extranuclear oxygen atom. It may be noted that the dissociated proton is normally required in formation of one of the hydrogen bonds between guanine and cytosine moieties of DNA according to the Watson–Crick model.



It appears, therefore, that at neutral *p*H the effect of alkylation of DNA at $N_{(7)}$ of guanine moieties would be to weaken the bonding between guanine and cytosine, since the tendency for the alkylated guanine to exist in the form shown in (I) would be much greater than for normal guanine. Furthermore, it is possible that during duplication of DNA the specificity of base-pairing would be weakened, since the number of hydrogen bonds which could form between alkylated guanine in form (I) and cytosine would be reduced by one. It may be noted that the configuration of (I) is such as to permit two hydrogen bonds to form with thymine, involving $N_{(1)}$ and the extranuclear amino group of the guanine moiety (Fig. 3). The configuration of this base-pair is such that it could fit into the DNA structure in place of a normal guanine-cytosine pair.

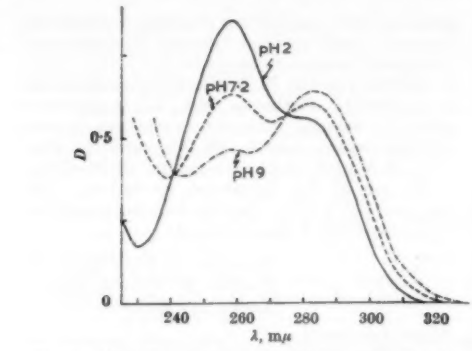


Fig. 2. Absorption spectra of 7-methylguanosine (0·0234 mgm./ml.) at *p*H 2, *p*H 7·2, and *p*H 9, 20°

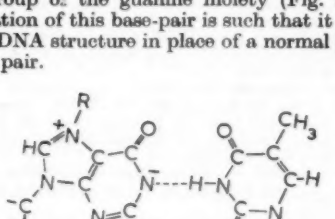


Fig. 3. Possible base-pairing of alkylated guanine and thymine

We suggest, therefore, that the mutagenic action of the alkylating agents could be due to their ability to alkylate DNA at $N_{(7)}$ of guanine moieties, thus strengthening the acidity of the —NH—CO— group in alkylated guanine so that its dissociation would cause a tendency for alkylated guanine to base-pair with thymine instead of cytosine during DNA duplication. After such anomalous base-pairing, in subsequent DNA duplications an adenine-thymine pair would replace the original alkylated guanine-cytosine pair.

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PHYSIOLOGY

A Central Action of 5-Hydroxytryptamine and Noradrenaline

THE experiments reported here were based on the observation that an intra-carotid or intravenous injection of *d*-lysergic acid diethylamide (LSD-25) depresses the transmission of orthodromic impulses through the lateral geniculate nucleus of the cat[1]. The nature of this depression suggests that LSD-25 can compete with the excitatory transmitter for post-synaptic receptor sites. Consequently the known antagonism between LSD-25 and 5-hydroxytryptamine (5-HT) at receptors of smooth muscle[2] indicates the possibility that the as yet unknown central excitatory transmitter might be related to 5-HT. It is probable that the failure to demonstrate an action of 5-HT on these neurones[1] was due to the blood-brain barrier, which is relatively impermeable to this substance[3].

In the present investigation, 5-HT and a series of structurally similar compounds, including some catecholamines, have been applied electrophoretically to single neurones in the lateral geniculate nucleus of a cat anæsthetized with pentobarbital. The direct extracellular application of drugs circumvents the blood-brain barrier and the actions of four different compounds could be compared on single cells by the use of five-barrel electrodes[4]. The spike responses, 0·2–1·0 mV. in amplitude and of negative–positive voltage sequence, were recorded by means of the central barrel of the electrode, which contained 4 *M* sodium chloride. Most substances were passed as cations from saturated aqueous solutions of *p*H 3–4. These included 5-HT creatinine sulphate, tryptamine, bufotenine, LSD-25, 2-bromo-LSD(BOL-148), psilocin[5], noradrenaline bitartrate, adrenaline bitartrate and 3-hydroxytyramine. L-Glutamic acid and psilocybin[5] were applied as anions from solutions of the sodium salt at *p*H 8.

When an electrophoretic current was used to apply cations from a saturated aqueous solution of 5-HT creatinine sulphate, there was suppression of the orthodromic responses of neurones which responded monosynaptically, and occasionally repetitively, to an optic nerve volley. Such was not the case when the electrode contained creatinine sulphate instead of the 5-HT creatinine sulphate complex, hence it is reasonable to assume that the depression observed with the complex was due to 5-HT. The latency of this effect was reciprocally related to the magnitude of the electrophoretic current and for currents of 20–100 mμamp. was about 10–2 sec. Following the termination of the current flow the spike responses recovered in 5–20 sec. When L-glutamate was applied from another barrel, the excitation of the neurones, measured by the frequency of the firing evoked by the amino-acid[4], was unaltered by 5-HT. Furthermore, the antidromic spike responses of these neurones, evoked by stimulation of the optic radiation beneath the ipsilateral gyrus marginalis of the cerebral cortex, were unaffected by 5-HT. These actions of 5-HT are illustrated in Fig. 1.

All the substances mentioned here, except creatinine and L-glutamic acid, had actions identical with those of 5-HT. Although it is difficult to compare various substances quantitatively by these electrophoretic techniques[4], an approximate estimate of the potency of the compounds can be given. 5-HT was the most effective depressant of these cells, as

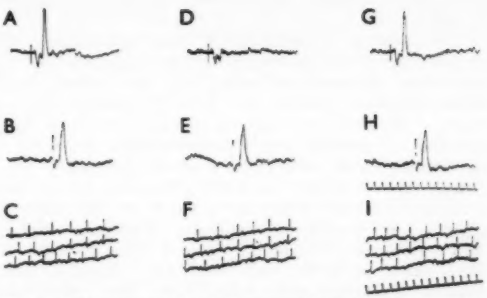

Fig. 1. Spike responses of a single neurone in the lateral geniculate nucleus responding monosynaptically to a contralateral optic nerve volley (*A*, *D*, *G*), antidromically to a stimulus to the ipsilateral optic radiation (*B*, *E*, *H*) and repetitively to L-glutamic acid (*C*, *F*, *I*) which was applied from one barrel of a five-barrel electrode using an anionic current of 100 mμamp. After the control responses *A* and *B*, a current of 20 mμamp. passed cations from an aqueous solution of 5-HT creatinine sulphate (*p*H 3·3) for 12 sec. The responses *D* and *E* were recorded 5–7 sec. after the current started and *G* and *H*, 5–7 sec. after it terminated. *C*, *F*, *I* were recorded with lower amplification (all spikes are approximately 0·5 mV. in amplitude) in another series: *C*, control; *F*, 31 sec. after a current of 20 mμamp. passed 5-HT creatinine sulphate into the environment of the cell. At this time orthodromic responses were suppressed. *I*, 2 min. after the 5-HT application ceased, the orthodromic responses having recovered fully. Time, msec. for *A*, *B*, *D*, *E*, *G*, *H* beneath *H*; 10 msec. for *C*, *F*, *I* beneath *I*

determined by the magnitude of the electrophoretic current necessary to block the excitation of single cells within a given time. The other agents were less effective, the descending order of potency being tryptamine; bufotenine; LSD-25, psilocin and psilocybin; 3-hydroxytyramine; noradrenaline and adrenaline; BOL-148. The latter compound had a very weak action. Several experiments were carried out to test whether BOL-148 interfered with the effect produced by 5-HT and no antagonism could be detected. In addition to being less effective than 5-HT, some of the other active compounds were more prolonged in their action after the applying current was terminated. Thus the recovery from an application of LSD-25 using an electrophoretic current which produced depression in 30–40 sec. often took 10–15 min.

Several conclusions can be drawn from these observations.

The failure of 5-HT to affect either the antidromic firing of these neurones or the firing evoked by L-glutamic acid indicates that 5-HT does not significantly modify the membrane conductance of the cells. Thus it is very unlikely that it was combining with the sub-synaptic receptors of inhibitory synapses and activating the associated receptor membrane, or that it was depressing the electrically excitable membrane of the cells in some other fashion. It is probable that the depression of the orthodromic responses was due to a reversible interaction between 5-HT and the receptors at excitatory synapses which are specialized for the transmitter released from the optic tract terminals. However, the possibility that 5-HT was depressing the release of this transmitter cannot be excluded until the transmitter is identified and applied to the synapses.

The antagonism which is exhibited between 5-HT and the other compounds, when tested on smooth muscle, was not apparent at these central synapses. Indeed these 'antimetabolites'[2] had actions similar to that of 5-HT. Presumably all these depressants interacted with the same receptors, the potency and the duration of action of a particular compound

being determined by the stability of the drug-receptor complex.

The possible interaction of these substances with post-synaptic transmitter receptors suggests that the excitatory transmitter acting on the lateral geniculate neurones might be related structurally to them. Experiments are proceeding with a large number of indoles and catecholamines in order to determine those structural features necessary for a compound to interact with the transmitter receptors.

Grateful acknowledgment is made for the following gifts. 5-HT creatinine sulphate, creatinine sulphate and bufotenine (Dr. M. E. Speeter, The Upjohn Co., Michigan), LSD-25 and BOL-148 (Sandoz, Ltd., Basle), psilocin and psilocybin (Dr. Hofmann, Sandoz, Ltd.).

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Hormones and Electrolyte Excretion in Sheep

Merino sheep in hot environments effectively reduce the rate of excretion of water but that of sodium and potassium may not change, and often increases during periods of dehydration[1]. Renal hæmodynamics may be concerned in this adjustment of kidney function but endocrine activity has been examined in the search for possible mechanisms.

Merino ewes were accustomed to life in metabolic cages on a fixed feeding regime at room temperatures between 22° and 24° C. Electrolyte excretion was followed throughout periods of 36 hr. using a fraction collector to take specimens at ½ hr. intervals. There was considerable variation in excretion, mainly following morning feeding which produced a rise in sodium and potassium output by day and a low rate of excretion at night. Sheep were therefore trained to take lucerne chaff during a period of 3 hr. between 10 a.m. and 1 p.m. It was observed that within 20–30 min. of beginning to feed, normally alkaline (*p*H 8·2–8·6) urine became acid (*p*H 5·2–6·6) for about 1 hr. While feeding continued (Fig. 1*c*) the urine returned to a *p*H of 8·2. Production of saliva with a high bicarbonate content could leave the blood depleted of alkali and an acid urine could result.

Infusion of small amounts of hormone intravenously over an hour was undertaken. Six hormones were examined. Mixed arginine and lysine vasopressin ('Pitressin'), infused at the rate of 12 milliunits/min., had two types of effect. At urine flows lower than 1 ml./min. there was an increase of water, sodium, potassium and chloride excretion per unit of time (Fig. 1*a*). At flows greater than 2 ml./min. there was a reduction of flow of urine and increased output of electrolyte. The osmolar excretion-rate increased at both high and low levels of urine flow under the influence of vasopressin. This occurred both in the field (the tropics at 21° S. latitude) and in the laboratory. During diuresis the osmolarity of urine was 100–200 m.osmol./l., and at low flows it was 1,400–1,800 m.osmol. Vasopressin produced urine between 500 and 1,700 m.osmol./l. always more concentrated than plasma.

Lysine vasopressin gave identical results. Although vasopressin does not produce the changes of urine flow found in a hot environment, it could contribute to the sodium excretion which can occur in such circumstances. At both high and low rates of urine flow, vasopressin increased glomerular filtration (measured by creatinine clearance) so that differences in renal dynamics do not account for the phenomena. It appears that the effect of the polypeptide is on electrolyte movements (mainly potassium) in the tubule.

Infusion of adrenaline at a rate of 15 μgm./min. into sheep with urine flows of 0·4–1 ml./min. (Fig. 1*b*) led to excretion of 3–6 ml./min. of water with no increase of electrolyte output. In partially dehydrated animals there was little effect on water flow. Noradrenaline infused at a rate of 5 μgm./min. increased water, sodium and potassium excretion. The effect of adrenaline in increasing water excretion is the opposite of that found during dehydration. Aldosterone increased the re-absorption of sodium in sheep given salt supplements (Fig. 1*c*) but it did not increase potassium excretion as in carnivores or man[2]. There was no change in *p*H and little in water output under the influence of an infusion of 10–300 μgm. *d,l*-aldosterone in the course of an hour.

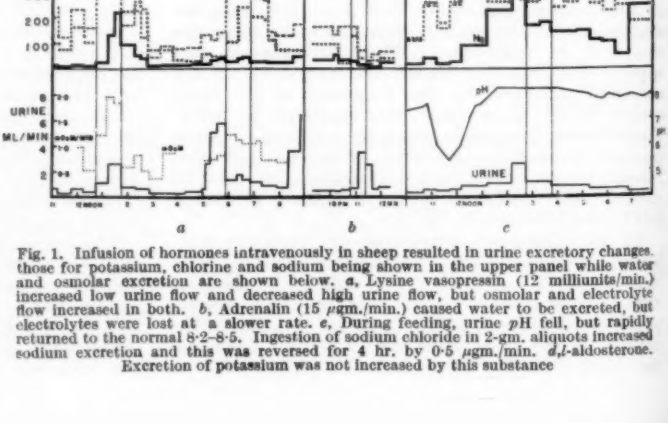

Fig. 1. Infusion of hormones intravenously in sheep resulted in urine excretory changes, those for potassium, chlorine and sodium being shown in the upper panel while water and osmolar excretion are shown below. *a*, Lysine vasopressin (12 milliunits/min.) increased low urine flow and decreased high urine flow, but osmolar and electrolyte flow increased in both. *b*, Adrenalin (15 μgm./min.) caused water to be excreted, but electrolytes were lost at a slower rate. *c*, During feeding, urine *p*H fell, but rapidly returned to the normal 8·2–8·5. Ingestion of sodium chloride in 2-gm. aliquots increased sodium excretion and this was reversed for 4 hr. by 0·5 μgm./min. *d,l*-aldosterone. Excretion of potassium was not increased by this substance

The sheep is thus different from most laboratory animals and man[3] in having a large acid shift of its urine during feeding; in increasing the excretion of salts and water during the action of vasopressin; and in showing no increased potassium excretion under the influence of aldosterone.

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Paradoxical Effect of Phlorizin on Glucose Uptake and Intracellular Accumulation of Amino-Acids in Rat Kidney Slices *in vitro*

THE discovery by von Mering[1] that phlorizin causes glycosuria in the dog and the observation that phlorizin prevents renal tubular re-absorption of glucose[2,3] have evoked considerable interest in this substance as a tool in the investigation of cellular transport mechanisms. Much work with phlorizin has shown this drug to be a potent inhibitor of penetration of hexoses and other sugars in gut preparations[4,5], kidney slices[6], ascites tumour cells[7], and red cells[8]. Significant alteration of intracellular metabolism has also been noted. This literature has recently been reviewed by Lotspeich[9] and Crane[10].

We have reported[11] that rat kidney cortex slices possess an active transport system for the intracellular accumulation of amino-acids against a concentration gradient. This system is dependent on oxidative metabolism as shown by the inhibition produced by anaerobiosis and dinitrophenol. In view of the known inhibition by phlorizin of sugar penetration and re-absorption in the kidney, experiments were carried out to ascertain whether this drug similarly affected the penetration of amino-acids into kidney cells. This communication demonstrates the unexpected finding that, in contrast to the inhibition of glucose uptake, phlorizin increases the intracellular concentration of amino-acids.

Renal cortical slices from male Sprague–Dawley rats were cut with a Stadie Riggs microtome. Following a 10-min. pre-incubation at 25° C. in isotonic Krebs–Ringer bicarbonate buffer, the slices (about 100 mgm./flask) were transferred to 25-ml. Erlenmeyer flasks containing the amino-acids labelled with carbon-14 in 2 ml. Krebs–Ringer bicarbonate buffer, pH 7·4. The flasks were gassed with 95 per cent oxygen, 5 per cent carbon dioxide, sealed and incubated in a Dubnoff metabolic shaker at 37° C. At the end of the incubation period the slices were dipped in isotonic saline, blotted, weighed on a torsion balance and placed in 2 ml. of boiling distilled water for 5 min. Aliquots of the incubation medium and the aqueous tissue supernatant were then counted in a liquid scintillation spectrometer, and the ratio of c.p.m. per ml. intracellular fluid to c.p.m. per ml. extracellular fluid calculated. Extracellular space was estimated using inulin labelled with carbon-14. Glucose disappearance from the medium was assayed with glucose oxidase.

The results of these experiments are shown in Table 1. Glucose uptake was reduced at phlorizin concentrations of 5×10^{-4} *M* and higher, reaching a 50 per cent reduction at 3×10^{-3} *M*. The ratio of intracellular to extracellular amino-acid concentration, however, was increased for all the amino-acids studied. The increase was most pronounced at the highest phlorizin concentration employed, the intracellular concentration of α-amino-isobutyric acid (AIB) being increased by 77 per cent at 3×10^{-3} *M*. The magnitude of the effect on basic amino-acids was somewhat less than that seen with neutral ones. For AIB the increase in this ratio was observed at the same concentration of the drug which was noted to block glucose uptake. Although the fact that the divergent effects on glucose and amino-acids occur in the same concentration-range suggests the two effects may be inter-related, the amino-acid effect is independent of the presence of glucose in the medium.

Table 1. EFFECT OF PHLORIZIN ON GLUCOSE UPTAKE AND INTRACELLULAR CONCENTRATION OF AMINO-ACIDS BY RAT KIDNEY SLICES. THE 90-MIN. INCUBATION WAS CARRIED OUT IN TRIPLICATE

Phlorizin* concentration (*M*)	Glucose† uptake (γ/100 mgm.)	Ratio of intracellular to extracellular amino-acid concentration: AIB‡	Glycine	L-Phenylalanine	L-Lysine	L-Histidine
None	258	3·49	5·76	1·85	3·52	2·28
10^{-5}	247					
5×10^{-4}		3·99				
10^{-4}	230	3·89				
5×10^{-4}	157	4·71				
10^{-3}	143	5·09	7·53	2·20		2·61
3×10^{-3}	129	6·18	9·49	3·42	4·55	3·41

* Phlorizin (Mann Co.) was twice recrystallized.
† Initial glucose concentration, 1 mgm./ml. (5·6 m*M*).
‡ Initial amino-acid concentrations ranged from 0·05 to 0·17 m*M*.

In contrast to the kidney slices, experiments with isolated rat diaphragm have demonstrated a slight reduction in the intra-cellular concentration of AIB at 3×10^{-3} *M* phlorizin. This has been reported by others[12]. Thus, increased intracellular amino-acid concentration as a result of phlorizin action is not a common property of all tissues.

Various components of the phlorizin molecule, the aglycone, phloretin, and phloroglucinol were also studied in order to see if the complete glycoside was needed to produce the effect on amino-acids. Neither phloretin nor phloroglucinol produced a similar increase in intracellular amino-acid concentration. Phloretin is known to be weakly active in inhibiting tubular reabsorption of glucose[13]. The complete glycoside appears to be necessary for both the glucose and the amino-acid effect.

The mechanism of action of phlorizin-induced inhibition of sugar penetration into cells is unknown although the general view is that the drug affects membrane permeability independently of the observed inhibitory effects on oxidative metabolism[14] as well as various enzymes[10]. The explanation for the amino-acid effect is unknown. Since these results are seen with a non-utilizable amino-acid (AIB), the phenomena cannot be related to inhibition of amino-acid metabolism *per se*. The intracellular concentration of the amino-acid is an equilibrium between influx and efflux rates. Phlorizin may produce the observed increased intracellular amino-acid concentration either by augmenting the transport-rate of amino-

acids into, or by inhibiting the transport rate out of, the cell. Phlorizin is known to inhibit the tubular excretion of organic acids[15], perhaps indicating a selective effect on amino-acid efflux-rates. However, an induced binding of amino-acids within the cell must also be considered as an explanation. The possible basis for the observed phenomenon is under investigation.

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Heart-rates and the Sub-lingual Temperatures of Heat-acclimatized People

People resident at sea-level in the equatorial tropics are considered to be 'naturally' acclimatized to heat[1]. There is evidence that the indigenous peoples of these regions have low forearm blood flows and systolic blood pressures in relation to their surface and deep-body temperatures[2–4]. This evidence seems to indicate that the cardiovascular systems of heat-acclimatized people are relatively insensitive to increases of body temperature. If this is true, the relationship between the heart-rates and sub-lingual temperatures of tropical people would be expected to differ from that of people who are not acclimatized to heat. The relationship between the heart-rates and sub-lingual temperatures of young English subjects, at rest in a temperate climate, has been investigated by Tanner[5]. Comparable data are not available for the indigenous peoples of the tropics.

In order to provide these data, measurements of heart-rates and sub-lingual temperatures have been made on thirty-two male Asian subjects in Singapore. The subjects were twenty-two Chinese, eight Indians and two Malays; their average age was 22·7 yr. (*S.D.* = ± 3·1) and their mean body-weight was 57·3 kgm. (*S.D.* = ± 7·2). Each subject wore his normal clothing[4] and lay on his back for 90 min. before any measurements were made. Heart-rates and sub-lingual temperatures were measured every 5 min. for 30 min. and the average of readings for each subject was taken. The mean environmental temperatures during all experiments were 28·9 ± 1·0° C., dry bulb, and 25·6 ± 0·9° C. wet bulb. Heart-rates were counted from electrocardiograms and sub-lingual temperatures were measured by means of a 40 s.w.g. copper-constantan thermocouple.

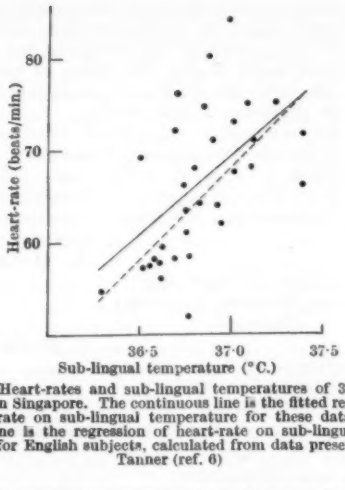

Fig. 1. Heart-rates and sub-lingual temperatures of 32 Asian subjects in Singapore. The continuous line is the fitted regression of heart-rate on sub-lingual temperature for these data. The broken line is the regression of heart-rate on sub-lingual temperature for English subjects, calculated from data presented by Tanner (ref. 6)

The heart-rates and sub-lingual temperatures of the thirty-two subjects are shown in Fig. 1. The correlation coefficient between heart-rate and sub-lingual temperature was 0·554 ($0·01 > P > 0·001$). The equation for the regression of heart-rate on sub-lingual temperature was:

$$\text{Heart-rate (beats/min.)} = (17·24 \times \text{sub-lingual temperature in °C.}) - 569·19$$

Also included in Fig. 1 is the regression line for heart-rate on sub-lingual temperature calculated from data presented by Tanner[6]. Tanner's data were obtained from forty-six English subjects whose mean age was 22·1 ± 4·1 yr.; measurements of heart-rates and sub-lingual temperatures on these subjects were made while they lay on a couch at a room temperature of 17·5–20·0° C. The correlation coefficient between heart-rate and sub-lingual temperature in the forty-six subjects was 0·510 ($P < 0·001$) and the equation for the regression line of heart-rate on sub-lingual temperature was:

$$\text{Heart-rate (beats/min.)} = (19·54 \times \text{sub-lingual temperature in °C.}) - 655·10$$

The heart-rates and sub-lingual temperatures of the heat-acclimatized people in Singapore tended to be higher than those of Tanner's subjects in England. The slope of the regression line of heart-rate on sub-lingual temperature was slightly greater for the English subjects. However, the correlation and regression coefficients for the two groups of data were not significantly different ($P > 0·10$).

These results have not provided any evidence that acclimatization to heat is associated with a significant general variation in the sensitivity to temperature of the tissues of the cardiovascular system. The possibility remains that changes of sensitivity to temperature are localized in discrete areas of the body such as the forearm blood vessels or centres in the brain. The latter possibility would be in agreement with a previous suggestion that acclimatization to heat

involves adaptive changes in the central nervous system[7].

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BIOPHYSICS

Elimination of the Liquid Junction by using Glass Electrodes

IT is assumed in measuring potential with hydrogen or alkali-sensitive glass electrodes[1] that the salt bridge which connects the reference electrode to the solution generates a negligibly small potential at the liquid junction. This is a reasonable assumption so long as the unknown solution contains no colloids, and its ionic strength is approximately that of the buffers used in standardization. Unfortunately these ideal conditions are seldom met in biological systems. The resultant error is often of a serious magnitude, as shown in experiments on *p*H in colloidal systems[2,3].

In many inorganic systems the liquid junction potential has been eliminated by the use of silver–silver chloride and other metallic reference electrodes placed directly into the unknown solution without a salt bridge[4]. The metallic reference electrodes function poorly in most biological systems because of the various interfering oxidizing and reducing substances which are present.

Glass electrodes are not affected by oxidation-reduction reactions and are well suited for use in biological media. With these advantages in mind, a system was designed and tested in which a hydrogen-sensitive glass electrode functioned as a reference electrode for sodium activity measurements with a sodium-sensitive glass electrode. The *p*H of the solution being tested was kept at 7·21. Any error in sodium determination which might have occurred if the *p*H were allowed to shift would tend to be automatically balanced out because the sodium-sensitive glass is also sensitive to hydrogen ions. The sodium concentration was varied by addition of 1 *N* sodium chloride to the buffer solution (*tris*-hydroxymethyl-aminomethane buffer[5]).

The sodium-sensitive glass electrode was of the bulb type (Electronic Instruments, Ltd., glass *BH* 104) while Corning 015 glass was fused to a lead glass stem to form the hydrogen-sensitive MacInnes–Dole type electrode. All potentials were measured with the Vibron model 33*B* electrometer and model *C*33*B* 'bucking' circuit.

Hydrogen ion sensitivity of the Corning 015 glass electrode was checked with buffers from the National Bureau of Standards (from *p*H 4·65 to 9·18), using a saturated potassium chloride–calomel reference electrode. The liquid junction between the saturated potassium chloride and buffer being tested was made through a porous ceramic plug. The sodium ion sensitivity of the *BH* 104 glass was tested in a *tris* buffer solution (*p*H 7·21) by varying the concentration of sodium with 1 *N* sodium chloride. The reference electrode was again the saturated potassium chloride–calomel with a porous ceramic plug. Representative data (circles) for the sodium-sensitive glass electrode and calomel reference are shown in Fig. 1. The hydrogen ion-sensitive glass electrode was then substituted for the calomel reference and the sodium ion measurements repeated in the *tris* buffer solutions (triangles in Fig. 1).

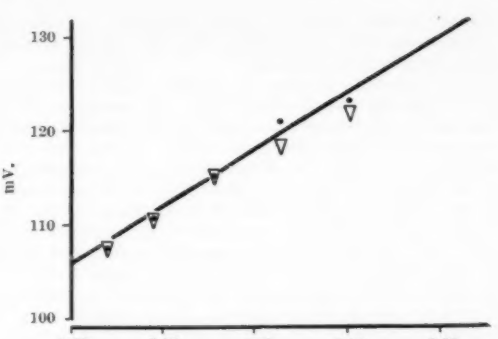

Fig. 1. Representative sodium-sensitive glass electrode potentials (circle) using a calomel reference electrode and (triangle) using a Corning 015 MacInnes–Dole glass electrode as the reference electrode. The straight line plots the slope of the theoretical potential given by the Nernst equation $E = E^{\circ} + 2{\cdot}303RT/F$ (log a_{Na^+}) while the points are experimental measurements. The value of $2{\cdot}303RT/F$ was taken as 59 mV. at 23° C. The negative logarithm of the activity of Na^+ is plotted on the abscissa using an activity coefficient of 0·73 based on the presence of a constant 0·2 *M* buffer solution

The equivalent response of the sodium ion electrode with either the standard saturated potassium chloride–calomel reference or the Corning 015 glass reference illustrates the ability of the glass electrodes to function in a system as both an ion-specific electrode and reference electrode. The simplicity of this method of eliminating the liquid function should prove useful to workers in many fields, especially when the specific cation to be measured is a solute in a complex solution.

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Mechanism of Nicotinic Action at the Adrenal Medulla: Calcium as a Link in Stimulus-secretion Coupling

IN experiments to probe the mechanism of transmission of sympathetic activity at the adrenal medulla we have found that acetylcholine, which is believed to be the chemical transmitter, evokes catecholamine secretion through some calcium-dependent process. Our evidence, obtained in experi-

ments in cats' adrenal glands perfused *in vivo* or *in vitro* with Locke's solution or variations of it, may be summarized as follows.

Acetylcholine failed to release catecholamines when calcium was omitted from the perfusion fluid (in similar experiments in which sodium or potassium was omitted acetylcholine still had a strong stimulant effect). The amount of catecholamines released by acetylcholine varied with the calcium concentration of the perfusion fluid over a wide range, being increased about 50 per cent above the normal with 8·8 m*M* calcium and being about doubled with 17·6 m*M* calcium. Calcium itself (2 m*M*) following a period of calcium-free perfusion caused a vigorous secretion of catecholamines. This evidence, considered along with the known ability of acetylcholine to increase membrane permeability at other junctional sites, led us to postulate that acetylcholine evokes secretion of catecholamines by causing an influx of calcium ions into the adrenal medullary cells[1].

In support of this postulate, one of us (W. W. D.) has recently found in experiments with Dr. A. M. Poisner that acetylcholine (10^{-5} gm./ml.) greatly increases the uptake of calcium-45 by the adrenal medulla. We have in fact proposed that calcium has a role in the process we have termed 'stimulus-secretion coupling' analogous to its supposed role in 'excitation-contraction coupling' in muscles. A full account of these experiments will be published elsewhere[2].

We have now obtained evidence that other substances which stimulate the adrenal medulla and which belong to the family of drugs with 'nicotinic' actions evoke secretion in a similar fashion. The present experiments were carried out on cat's adrenal glands acutely denervated and perfused *in situ* through the aorta with Locke's solution. In such experiments the 'resting' secretion of catecholamines appearing in the effluent collected from the adrenal veins was usually equivalent to about 50–100 mμgm. adrenaline (base)/min. The addition of 10^{-5} gm./ml. nicotine, tetramethylammonium, lobeline or dimethyl-4-phenylpiperazinium iodide (DMPP) to the perfusion fluid caused an enormous increase in the catecholamine output. Not uncommonly the secretion evoked by these drugs, particularly DMPP and nicotine (which seemed to be especially active), was more than 100 times the control 'resting' secretion.

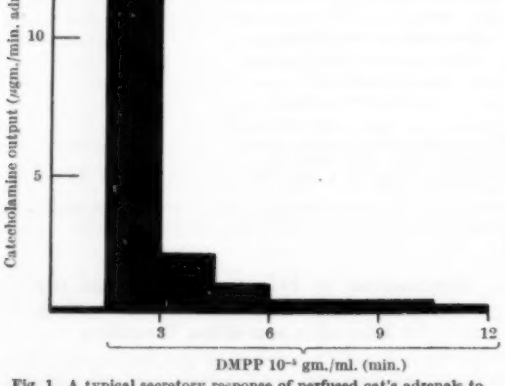

Fig. 1. A typical secretory response of perfused cat's adrenals to DMPP 10^{-5} gm./ml. Both adrenals were perfused with Locke's solution and the effluent assayed in terms of adrenaline (base) by bioassay with the spirally cut rabbit aorta. Before addition of DMPP the gland was perfused for 20 min. during which the 'resting' output was determined: the output in the last 1½ min. of this period is indicated

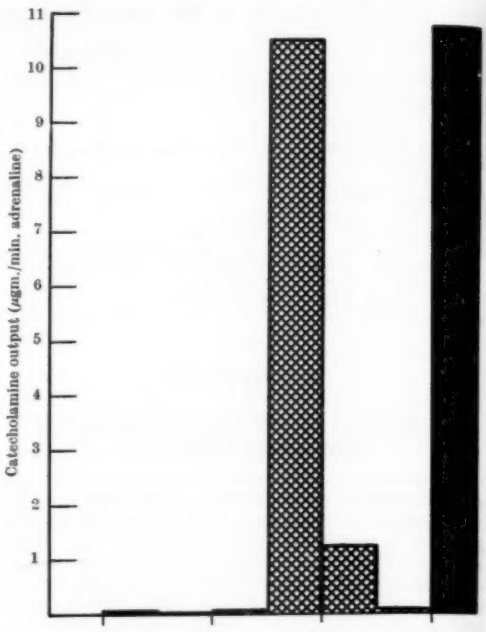

Fig. 2. Failure of nicotine (10^{-5} gm./ml.) to evoke catecholamine secretion in the absence of calcium. The thickening of the base-line to the left indicates the 'resting' outputs (min. 4–12) and the negligible response to nicotine (min. 12–16) in calcium-free Locke's solution. The cross-hatched bars which follow indicate the stimulant effect of re-introducing calcium (in the form of Locke's solution). The solid bar to the right indicates the control response to nicotine in Locke's solution

A typical response is shown in Fig. 1, from which it can be seen that DMPP increased the rate of secretion to more than 13 μgm./min. This high rate of secretion was not maintained, but fell progressively so that in about 10 min. it was near to the resting value despite the continued presence of the drug. The cause of this progressive diminution in the response was not determined: for the present purpose tests were made with each of these substances by perfusing them in this same concentration (10^{-5} gm./ml.) for periods of 4 min. In each experiment one or the other drug was first tested in Locke's solution free of calcium and then in Locke's solution. In each instance the drugs were active in Locke's solution but inactive (or almost so) in calcium-free Locke's solution. A typical experiment is shown in Fig. 2. After a period of perfusion with calcium-free Locke's solution (usually 16 min.) perfusion was switched to calcium-free Locke's solution with the addition of nicotine (10^{-5} gm./ml.). This raised secretion very little, if at all, above the resting value. Perfusion was then switched back to Locke's solution. This change in itself caused a transient but very vigorous secretion which, as mentioned above, is due to the re-introduction of calcium (it occurs also without

prior exposure to any of the stimulant drugs). When spontaneous secretion had returned to a low level perfusion was once more switched, this time to Locke's solution plus nicotine. Nicotine then exerted its familiar powerful excitant effect raising secretion to more than 10 μgm./min. Similar results were obtained with TMA, lobeline and DMPP.

It thus appears that these various drugs of the 'nicotine group' may all evoke catecholamine secretion by causing calcium ions to penetrate the medullary cells.

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PHARMACOLOGY

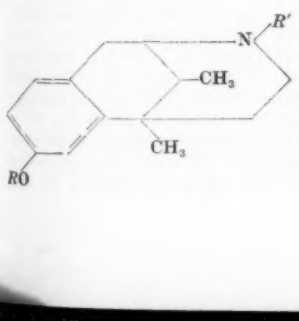
A New Potent Analgetic Antagonist

The discovery of a new potent analgetic with a reduced liability to addiction[1] in the benzomorphan series, namely, 2′-hydroxy-5,9-dimethyl-2-phenylethyl-6,7-benzomorphan[2] (I), has suggested further work in the series. 2′-Hydroxy-5,9-dimethyl-2-allyl-6,7-benzomorphan (III) is a potent antagonist of phenazocine, morphine, meperidine, and other analgetic agents. This compound is of special interest in that combinations of phenazocine and III in ratios of 5 : 1 to 100 : 1 retain analgetic activity in animals yet seem to be relatively devoid of physical dependence properties in the monkey in both acute and chronic experiments (biological details of this work will be described elsewhere).

The demethylation of 2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan has been described[2]. Reaction of the nor-compound (II) with a slight excess of allyl bromide in ethanol containing sodium bicarbonate for 18 hr. under reflux gave, after filtration, concentration and trituration with ether, the product (III), m.p. 141–144° in 46·5 per cent yield. Anal. calc.: $C_{17}H_{23}NO$; C, 79·33; H, 9·01; N, 5·44. Found: C, 79·06, 78·99; H, 9·19, 9·29; N, 5·64, 5·78.

A mixture of 5,9-dimethyl-2′-methoxy-6,7-benzomorphan (IV), prepared by treating 5,9-dimethyl-2′-hydroxy-6,7-benzomorphan[2] (II) in ethanol with diazomethane in ethanol containing sodium bicarbonate was treated with allyl bromide under reflux. After appropriate working up, the residue was dissolved in acetone and treated with ether to give the desired product (V), m.p. 216–218°, for the hydrochloride salt. Anal. calc. for $C_{18}H_{23}NO.HCl$: C, 70·22; H, 8·51; N, 4·55. Found: C, 70·00; H, 8·50; N, 4·78.

The present antagonist (III) combined with phenazocine in a 1 : 1 ratio showed no analgesia, and precipitated abstinence phenomena in the morphine-addicted monkey (Deneau, G. A., and Seevers, M. H., private communication). With a ratio of 9 parts of phenazocine to 1 of the antagonist (III), the analgesic effect was as good as with phenazocine alone, and there was a precipitation of abstinence in addicted monkeys as seen with nalorphine, instead of a suppression. Mixtures of 25 : 1 and 50 : 1 acted similarly; with a 100 : 1 mixture there was no precipitation of abstinence and no suppression up to a dose of 16 mgm./kgm. The retention of good analgesic effect without suppression of abstinence, indeed with the presence of nalorphine-like effects with respect to precipitation of abstinence in morphine-addicted animals, seems very promising and these mixtures will be studied in man. The antagonist (III) itself showed no significant analgesic effect in animals.



I R=H, R'=$CH_2CH_2C_6H_5$
II R=H, R'=H
III R=H, R'=CH_2—CH=CH_2
IV R=CH_3, R'=H
V R=CH_3, R'=CH_2CH=CH_2

An analgesic effect of nalorphine in man, and retention of the analgesic effect of morphine in appropriate mixtures of morphine and this antagonist, were first reported by Beecher and Lasagna[3]. Later it was shown that morphine–nalorphine mixtures administered repeatedly to former addicts (Fraser, H. F., and Isbell, H., personal communication) produced a milder degree of physical dependence than equivalent doses of morphine alone. N. B. Eddy, M. Piller, L. A. Pirk, O. Schrappe and S. Wenda[4] have shown that when mixtures of morphine and levallorphan (an antagonist of the morphinan group) were administered for chronic pain there was a definite trend toward delay in the development of tolerance and physical dependence under clinical conditions.

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Anti-Asthmatic Effect of Phenazone and Amidopyrine

Many chronic asthmatics state that they obtain relief from proprietary asthma powders available in Germany without medical prescription. Most of these powders contain either phenazone or amidopyrine, besides much smaller quantities of various other ingredients. In the list of German proprietaries (the "Red List"), we have found 53 such preparations, of which 27 contained phenazone, 22 amidopyrine and 4 both.

As little seems to be known about the anti-asthmatic effects of phenazone and amidopyrine, we have investigated the question in 46 patients with chronic asthma, who were trained in spirometry. In each experiment the patient's vital capacity was recorded successively three or more times, and he was then given placebo or drug by mouth. 1 hr. later a second series of vital capacities was recorded as before. The

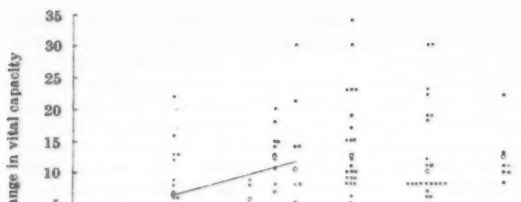

Fig. 1. Percentage change in vital capacity for placebo and doses of amidopyrine and phenazone. ●, Individual experiments; ○, mean response at each dose

means of vital capacities before and after treatment were calculated and results expressed as percentage change of vital capacity. On some occasions we also recorded peak flow and forced expiratory volume; but recording the latter tended to increase bronchial spasm.

Twenty-seven patients received phenazone in doses of 0·5, 1·0 or 2·0 gm. in 63 experiments, and 26 patients received amidopyrine in doses of 0·6, 1·2, 1·5 or 1·8 gm. in 43 experiments. Placebo was given in 17 experiments. The percentage changes in mean vital capacity from the basal values before treatment in each experiment and the overall mean values after placebo and at each dose of drug are plotted in Fig. 1. A representative example of the phenazone effect is given in Fig. 2.

Compared with the basal values before treatment, the increases in vital capacity after amidopyrine or phenazone are evident from Fig. 1; while the change after placebo is negligible. The differences between placebo and all doses of phenazone or of amidopyrine are highly significant ($P < 0{\cdot}001$ in both cases).

The calculated regression line for amidopyrine, which has significant slope ($P < 0{\cdot}05$), is also given in Fig. 1. A regression line was not calculated for phenazone, because it clearly would not have a significant slope. Where forced expiratory volume was recorded we found no definite effect; but the peak flow usually increased when the vital capacity increased.

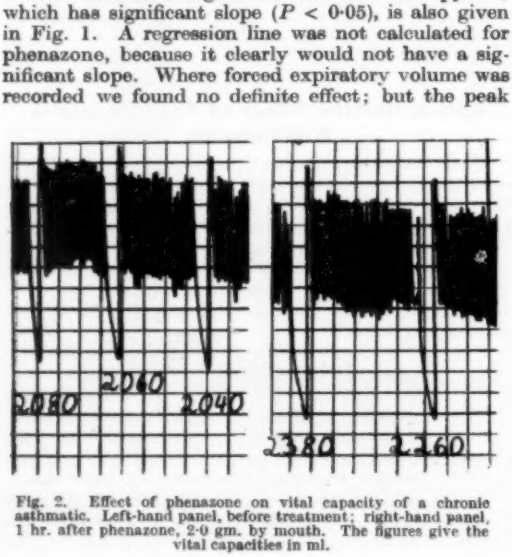

Fig. 2. Effect of phenazone on vital capacity of a chronic asthmatic. Left-hand panel, before treatment; right-hand panel, 1 hr. after phenazone, 2·0 gm. by mouth. The figures give the vital capacities in ml.

These results show that phenazone and amidopyrine lessen respiratory obstruction in most chronic asthma patients examined. Results with aspirin, so far unpublished, have been comparable. Our experience with other agents, such as histamine and isoprenaline, has shown that an increase or decrease of vital capacity by less than 4 or 5 per cent remains subjectively unnoticed, but greater changes are felt by the patient. Since most increases of vital capacity exceeded 5 per cent after all doses of both drugs, most patients felt relief from phenazone and amidopyrine, as was to be expected.

A search in the literature has so far revealed no previous reference to the anti-asthmatic action of amidopyrine. Two reports, one of 1889 [1] and one of 1890 [2], have been found on phenazone. In each of these the drug was claimed to have relieved asthma in one patient. Our results indicate that in many asthma cases both drugs play a more active part than was hitherto supposed.

The anti-asthmatic action of phenazone, amidopyrine and aspirin, which also inhibit histamine release in anaphylaxis[3], is particularly interesting because these drugs specifically antagonize bradykinin broncho-constriction in the guinea pig[4,5], and because inhalation of bradykinin aerosol reduces the vital capacity of chronic asthmatics[6]. We are therefore investigating the possible relationship between bradykinin and asthma; but in 30 experiments in which one of these analgesic-antipyretic drugs was given orally before the patient inhaled a bradykinin aerosol, the reduction of vital capacity by bradykinin was inhibited on only seven occasions. This result points against the antagonistic effect of these drugs to bradykinin in man, but work on this problem continues. Results will be published in detail later.

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PATHOLOGY

A New Method for Local Chemical Analysis of Human Tissue

As a consequence of the original work of Castaing[1] on the electron beam micro-analyser, widespread application has been made in the field of physical metallurgy[2]. It is the purpose of the present communication to record the applicability of this technique to biological problems. In the Castaing instrument a beam of electrons, focused to a spot 1μ in diameter, excites fluorescent or secondary X-rays in the specimen, which are analysed in a crystal spectrometer with Geiger counter detection. An optical microscope with a reflecting objective permits simultaneous viewing and analysis of the

irradiated spot. Mechanical stage motion allows the selection for analysis of any desired spot in the specimen. The range of elements at present detectable coincides with that of modern X-ray spectrographs, that is, elements lying above sodium in the Periodic Table. Two characteristics of these instruments of paramount importance to biological research are: (1) a detectable limit as low as 10^{-14} gm.; (2) spatial resolution of approximately 1μ. Birks has recently discussed the electron probe instrument in its numerous modifications[3].

In solids which are good conductors of heat and electricity, electron bombardment in the instrument produces no damage. In biological materials, however, damage to a specimen may be catastrophic under irradiation by 25-kV. electrons at current densities of the order of 100 amp./cm.^{2}. Our experiments have been made on microtome sections of tissue nominally 4μ thick, mounted on polished carbon rods. Sections were cut from formalin-fixed paraffin blocks, and picked up from distilled water with the carbon rods. Subsequent dehydration and removal of paraffin with organic solvents was accomplished, producing a specimen of tissue firmly bonded to the underlying carbon. These were analysed without further manipulation in the electron probe instrument manufactured by the Paris firm of CAMECA. Although produced by techniques standard in pathology, the specimens proved to be virtually indestructible.

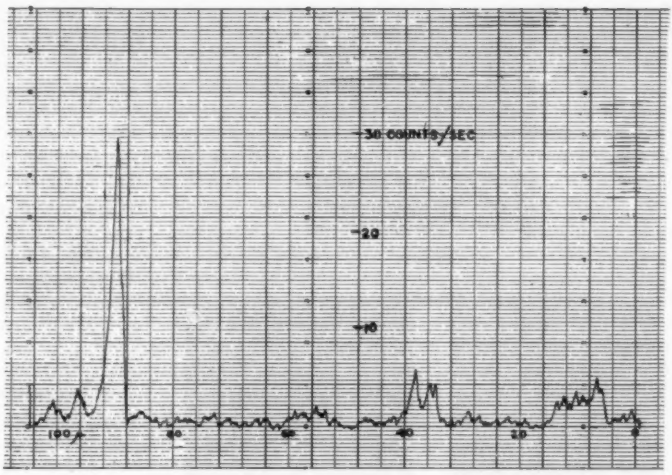

Fig. 1. Iron distribution in a synovial membrane

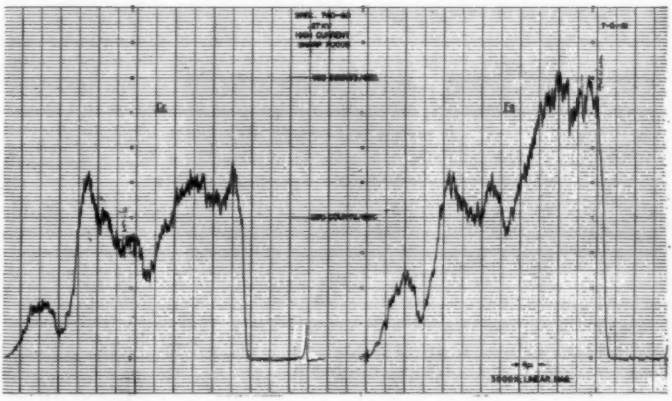

Fig. 2. Iron and chromium distribution in joint capsule (hip joint prosthesis)

Fig. 1 shows the results of an iron analysis in human synovial membrane, pathologically diagnosed as pigmented villonodular synovitis. The curve is a typical linear scan across the specimen, showing the intensity of iron K_α X-rays as a function of position. Beam current was 2×10^{-9} amp. at an accelerating potential of 27 kV. Sharp beam focusing is indicated by the steepness of the response curves. Maximum X-ray intensity observed in Fig. 1 is some 30 counts/s.; under identical instrument conditions a solid iron specimen emits 700 c.p.s. Under much higher beam currents this specimen of tissue was found to yield intensities from iron-rich regions as high as 800 c.p.s., with a spot diameter about twice as large as that used in obtaining Fig. 1. Therefore it may be estimated that the minimum detectable amount of iron in a spot of 4μ diameter is about one-thousandth of that actually present in this section of tissue.

An example of exogenous material present in tissue is furnished by the curves of Fig. 2. This material is a section of joint capsule taken from the vicinity of a surgical metallic implantation (hip joint prosthesis). Two linear scans show the distribution of iron and chromium along the same path. The remarkable similarity of these curves implies the co-precipitation of these two elements in this particular region of the tissue. Noteworthy is the low background, essentially zero, and the steepness of rise at the edge of the metal-rich areas. It was observed further that sequential beam traverses over the same path gave practically identical responses, that is, for analytical purposes the method is non-destructive under the instrumental conditions used (27 kV., 10^{-7} amp.; beam scanning speed, 12 μ/min.).

The same specimen was investigated in a scanning probe which utilizes the 'flying spot' arrangement of Cosslett[4]. In this instrument the electron beam is caused to execute two-dimensional scanning while the emitted X-rays are used to modulate the intensity of a synchronously driven cathode-ray tube. Photography of the cathode tube display yields a television-like image of the distribution of a chosen element. Alternatively, an image formed by back-scattered electrons may be displayed, giving information analogous to the conventional image formed by an optical microscope. Fig. 3 shows the same specimen as Fig. 2, imaged successively by means of scattered electrons, iron K_α, and chromium K_α X-rays. Identical areas at a magnification of 300 are covered by the three images. Also shown are two linear

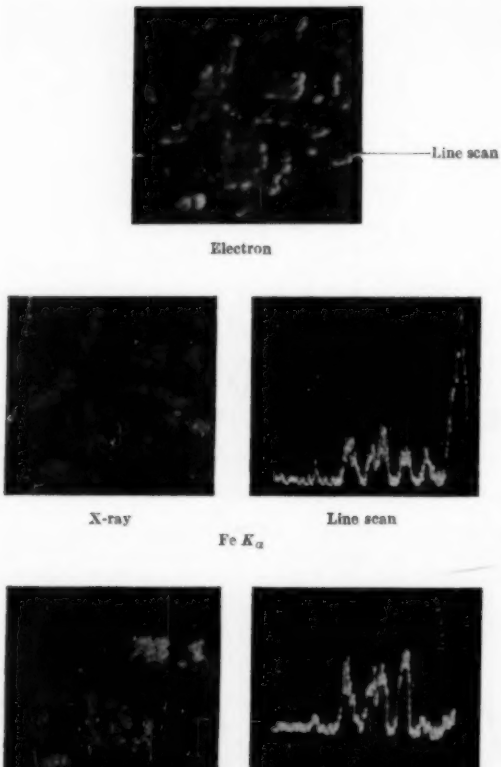

Fig. 3. Same specimen as Fig. 2, imaged by back-scattered electrons, iron and chromium X-rays

scans similar to those of Fig. 2, plotting variations in iron and chromium concentrations across the tissue. Fig. 3 clearly indicates differing composition from one metal-rich area to another in this tissue. No serious damage to the specimen occurred during the experiments, and the pictures of metallic localizations in Fig. 3 were reproducible.

It appears that the Castaing electron probe microanalyser is directly applicable to the field of human pathology. With proper specimen preparation, the microtome section is amenable to extremely sensitive chemical analysis. Many problems in cellular chemistry and pathology could be investigated by the use of the technique. For example, preliminary investigation has shown the feasibility of measuring the distribution of calcium and phosphorus in undecalcified bone blocks.

We wish to acknowledge with thanks the support and encouragement of F. L. LaQue, and the help of Carl Kruse, Cambridge Instrument Co., in the use of the beam scanning probe.

Note added in proof. An oral presentation in this subject was made on November 15, 1961, at the Eastern Analytical Symposium, New York City, entitled "Elemental Analysis (Cu, Ag, Fe) at the Subcellular Level in Tissue Sections of Human Specimens by Means of the Electron Microprobe", by A. J. Tousimis, George Washington University, and I. Adler, U.S. Geological Survey.

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Carcinogenicity of Dibenzo(a,l)pyrene

Dibenzo(*a*,l)pyrene (1,2,3,4-dibenzopyrene) has been shown to be present in cigarette smoke condensate and in automobile exhaust gas in small quantities[1–5]. In view of this, the relative dearth of tests for the carcinogenic activity of this substance is surprising. Though dibenzo(*a*,l)pyrene was regarded as a strong carcinogen by Cook and Kennaway[6] and by Pullman and Pullman[7], the only biological evidence we could find in the literature has been presented by Bachmann *et al.*[8]. These authors placed a 0·3 per cent dibenzo(*a*,l)pyrene solution in benzene on two mice. After 6 months one mouse died. The remaining mouse lived for 262 days and had developed an epithelioma. To our knowledge no other group of workers has reported the carcinogenic activity of dibenzo(*a*,l)pyrene[9].

This report presents results of testing the carcinogenic activity of different concentrations of dibenzo(*a*,l)pyrene. With slight modifications in the method, dibenzo(*a*,l)pyrene was synthesized by Zander's method[10]. The material used for testing was purified by vacuum-sublimation, repeated column chromatography on alumina and recrystallization from benzene. The purity of this hydrocarbon (m.p. 224–226° C.) was checked by ultra-violet absorption and fluorescence spectra and by paper chromatography[1].

Acetone solutions in concentrations of 0·25, 0·1, 0·05 and 0·01 per cent were applied thrice weekly to the backs of 6-week-old Swiss (Millerton) female mice. The materials were applied with one stroke of a camel-hair brush (No. 5). Re-grown hair was removed with an Otis clipper whenever necessary.

Table 1. Carcinogenic Activity of Dibenzo(*a*,l)pyrene

No. of months	0·25 per cent S*	P†	C‡	0·1 per cent S	P	C	0·05 per cent S	P	C	0·01 per cent S	P	C
1	20	0	0	20	0	0	20	0	0	20	0	0
2	20	0	0	20	0	0	20	0	0	20	0	0
3	20	0	0	20	0	0	20	0	0	20	0	0
4	20	5	0	20	5	0	20	0	0	20	0	0
5	17	65	30	20	35	10	20	15	5	20	0	0
6	10	95	50	13	75	35	18	65	10	20	5	0
7	0	100	100	6	95	70	3	95	65	18	20	10
8				3	100	85	1	100	85	16	35	20
9				0	100	95	0	100	100	12	50	35
10										8	75	55
11										2	85	85
12										0	85	85
Average conversion time in (days) from papilloma to carcinoma	21			27			29			35		
Tumour potency (ref. 11)	70			63			57			39		

* Survivals; † papillomas; ‡ carcinomas

The results of the experiment are listed in Table 1 and show carcinogenic activity for all concentrations tested. Comparing Blanding potency numbers[11] with previous data for benzo(*a*)pyrene 0·05 per cent, 94 (ref. 12), 0·01 per cent, 57 (ref. 13), and for dibenz-(*a*,*h*)anthracene 0·1 per cent, 48 and 0·01 per cent, 33 (ref. 13) shows that the carcinogenic activity of dibenzo(*a*,l)pyrene lies between that of the other two carcinogens.

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RADIOBIOLOGY

Production of Chromatid Aberrations by Diepoxybutane and an Iron Chelator

RECENT investigations have shown that chromatid breaks produced by certain radiomimetic agents in combination do not interact to produce reunions[1]. Specifically, breaks produced by 8-ethoxycaffeine do not form exchanges with those produced by maleic hydrazide, nor do the breaks produced by potassium cyanide and β-propiolactone interact with each other. Conversely, breaks induced by X-rays will interact with those produced by potassium cyanide, β-propiolactone, 8-ethoxycaffeine and maleic hydrazide. The interpretation of these results was stated in terms of differences in the bonds broken by the chemical agents, such differences precluding interaction or rejoining of broken ends of chromosomes.

As part of an investigation of radiomimetic agents and an iron chelator, a series of experiments utilizing combined treatments was performed. In addition, combined treatments with X-rays and 1,2-3,4 diepoxybutane or 2,2′ bipyridine were carried out. The material used consisted of the lateral roots of *Vicia faba* (Carter's Seville Longpod) grown at 20° C. for 7 days prior to treatment. The chemical agents were used as follows: 2,2′ bipyridine, 2×10^{-3} *M* for 15 min.; potassium cyanide, 1×10^{-3} *M* for 30 min.; 1,2-3,4 diepoxybutane, 3×10^{-3} *M* for 15 min. In the X-ray experiments, the roots were exposed to 100 r. X-rays administered in 54 sec. The source used was a 'Keleket 220–*C*' X-ray unit operated at 180 kV.p. and 18 m.amp. with 1·3 mm. aluminium and 0·8 mm. copper filtration. Irradiations were performed by Dr. John E. Edwards, of the Department of Physics, Ohio University. Roots were immersed in 1,2-3,4 diepoxybutane or 2,2′ bipyridine for 13 min. prior to irradiation, exposed while immersed, and rinsed 1–2 min. following exposure. Feulgen squashes were prepared from roots fixed 24 and 48 hr. following treatment.

Table 1. INTERACTION EFFECTS OF POTASSIUM CYANIDE AND 1,2-3,4 DIEPOXYBUTANE

Treatment	Recovery period (hr.)	Isochromatid deletions per cell*	Exchanges per cell Observed	Calculated Additive effect	Calculated Complete interaction
Potassium cyanide	48	0·24 ± 0·02	0·07 ± 0·01	—	—
Diepoxybutane	48	0·54 ± 0·01	0·20 ± 0·01	—	—
Potassium cyanide + diepoxybutane	48	0·82 ± 0·04	0·48 ± 0·03	0·27	0·51
Potassium cyanide and diepoxybutane†	48	1·57 ± 0·08	1·03 ± 0·06	0·27	0·51

* A minimum of 200 cells was analysed for each treatment.
† Simultaneously.

Table 2. PRODUCTION OF CHROMATID ABERRATIONS BY X-RAYS, 1,2-3,4 DIEPOXYBUTANE AND 2,2′ BIPYRIDINE

Treatment	Recovery period (hr.)	Isochromatid deletions per cell*	Exchanges per cell Observed	Calculated Additive effect	Calculated Complete interaction
Diepoxybutane	24	0·33 ± 0·01	0·11 ± 0·05	—	—
X-ray	24	0·26 ± 0·02	0·08 ± 0·01	—	—
Diepoxybutane + X-ray	24	0·62 ± 0·04	0·20 ± 0·02	0·19	0·39
Diepoxybutane	48	0·54 ± 0·01	0·20 ± 0·01	—	—
X-ray	48	0·13 ± 0·02	0·05 ± 0·01	—	—
Diepoxybutane + X-ray	48	0·68 ± 0·04	0·24 ± 0·02	0·25	0·46
2,2′Bipyridine	24	0·07 ± 0·005	0·01 ± 0·002	—	—
X-ray	24	0·26 ± 0·02	0·08 ± 0·01	—	—
2,2′ Bipyridine + X-ray	24	0·20 ± 0·02	0·10 ± 0·02	0·09	0·15
2,2′ Bipyridine	48	0·05 ± 0·006	0·01 ± 0·002	—	—
X-ray	48	0·13 ± 0·02	0·05 ± 0·01	—	—
2,2′ Bipyridine + X-ray	48	0·13 ± 0·02	0·04 ± 0·01	0·06	0·11

* A minimum of 200 cells was analysed for each treatment.

The peak effect of combined treatment with potassium cyanide and 1,2-3,4 diepoxybutane occurs in early interphase as indicated in the roots fixed 48 hr. after treatment. This result is consistent with my preliminary investigations. The combined applications of potassium cyanide and 1,2-3,4 diepoxybutane have elicited two unexpected results (Table 1). When the two agents were administered one following the other, in either order, the resultant frequency of exchanges clearly indicated interaction between the breaks produced by the two compounds. The implication is that the breaks produced are of a similar or identical chemical nature. This result is contrary to that found with potassium cyanide and beta-propiolactone[1]. The other unforeseen result arises from the simultaneous treatment with potassium cyanide and 1,2-3,4 diepoxybutane. The aberration frequencies were so high as to make analysis difficult. It is probable that in this case potassium cyanide as a metabolic inhibitor prevents the restitution of broken ends of chromosomes. Thus, all the breaks produced by the two agents are available at the same time and for a longer period of time favouring reunions and deletions. Potassium cyanide is known to inhibit the terminal stages of cellular respiration, and in this way reduces the available energy necessary for restitution[2]. Results of this type were also obtained in *Allium* roots treated with X-rays and carbon monoxide in the dark[3].

The results of the X-ray experiments are presented in Table 2. The frequencies of exchanges and isochromatid deletions produced by treatment with X-rays and 1,2-3,4 diepoxybutane indicate a strictly additive effect. This result is expected for the deletions but not for exchanges. Earlier work has indicated that, for the most part, breaks produced by radiomimetic agents interact with those produced by X-rays[1,4,5]. An explanation of these results may lie in the difference in the peak time of effect of the two

agents. Early interphase is the most sensitive period with respect to 1,2-3,4 diepoxybutane activity, whereas late interphase appears to be more sensitive to X-ray treatment[5]. Therefore, the majority of breaks produced by the two agents would not be available at the same time to facilitate reunions. This argument has been presented previously by Auerbach[4]. The additive effect produced by X-rays and 1,2-3,4-diepoxybutane is evident for both 24- and 48-hr. recovery despite the fact that 1,2-3,4 diepoxybutane has an appreciable effect in late interphase (Table 2, 24-hr. recovery period). This is also the time during which X-rays have their maximum effect. Presumably, a large number of broken ends are available at this time for interaction, but interaction does not occur. Since X-rays have an essentially random effect in producing chromosome breaks, certain of these breaks must involve bonds similar to those broken by 1,2-3,4 diepoxybutane as well. These breaks of similar bonds would be expected to undergo reunions with each other to increase the total yield of exchanges. The effect, therefore, cannot be explained in terms of one mechanism, but probably depends on considerations of both a temporal nature and a qualitative difference between the breaks produced by the two agents.

The results of the X-ray investigations with bipyridine (Table 2) are similar to those with 1,2-3,4 diepoxybutane. There is no enhancement of X-ray effect with 2,2′ bipyridine, nor is there any indication of interaction of breaks produced by the two agents. The effect is additive for isochromatid deletions and exchanges at both recovery periods. The result is in basic agreement with similar work by Kihlman[7]. Normally, one would expect at least an additive effect of the two agents for both aberration types. In the results reported here, even an additive effect is lacking in most instances. This may indicate that the effect of 2,2′ bipyridine is to reduce the total yield of aberrations in some manner. The sticky effect and clumping of chromosomes produced by 2,2′ bipyridine may cause a masking of the true breakage frequencies and may account for the apparent lack of an additive effect. This aspect of the results requires further investigation before a clear explanation can be presented. The absence of interaction between 2,2′ bipyridine and X-ray-induced breaks is again contrary to previous results. 2,2′ bipyridine appears to act over a long period during interphase, part of this period coinciding with the time during which the peak effect of X-rays occurs. Thus, the presence of breaks at the same time, and the random effect of X-rays, would be expected to permit interaction. The mechanisms of breakage by the two agents are possibly different or there may be a qualitative difference in the breaks produced. The low frequency of aberrations produced by 2,2′ bipyridine alone further complicates the situation, and the role of 2,2′ bipyridine as a metabolic inhibitor is even more difficult to evaluate from these results.

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Radioactivity in Wool

Kulwich *et al.* recently reported[1] the presence of unidentified γ-ray activity in several samples of wool. The bulk of the observed activity in the wool was shown to be most probably associated with its impurities and hence due to natural and fall-out contamination.

In May 1960 we examined a sample of wool for its potassium-40 content in conjunction with an experiment for the determination of lean-to-fat ratio *in vivo* by determining the potassium content of the animal. Since then, we have again examined two samples of raw wool for their associated radioactivity. Each sample consisted of approximately 500 gm. of the material, and the work was performed in a low-background room (iron room) of the Biological and Medical Research Division of the Argonne National Laboratory. A 5 in. × 4 in. sodium iodide (thallium) crystal detector was used in conjunction with an Argonne type 256-channel pulse-height analyser.

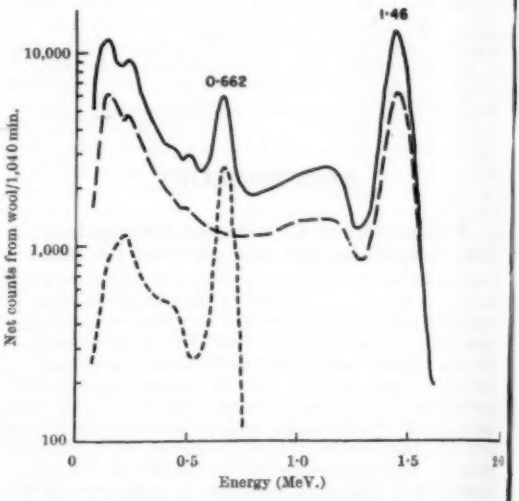

Fig. 1. Net γ-ray spectra of raw wool samples (——), compared with spectra for potassium-40 (– – –), and cæsium-137 (·····) standard

Our work confirms the presence of γ-emitting radionuclides in the wool of Hampshire ewe and Rambouillet ram lambs, and allows tentative identification of the major components of such activity. Fig. 1 shows the net γ-ray spectrum of one of the two wool samples as well as γ-ray spectra of potassium-40 and cæsium-137 for comparison. The spectra from the two samples of wool are identical in shape but differ in absolute amount of the activity present. Except for trace quantities of other nuclides only two prominent nuclides are observed, namely, potassium-40 and cæsium-137. In practice, the sample to be encountered probably will vary widely in activity, hence our values shown in Table 1 indicate only the order of magnitude to be expected.

Since no natural activity aside from that of potassium-40 was observed, one can conclude that the potassium-40 activity is present in wool grease, suint,

Table 1

	Hampshire ewe	Rambouillet ram
gm. potassium/kgm. wool	32	14
$\mu\mu$c. ^{137}Cs/kgm. wool	356	438
$\mu\mu$c. ^{137}Cs/gm. potassium	11	31

and dead skin in the wool[3]. Cæsium-137, on the other hand, probably comes from two different sources, namely, from fall-out contamination as well as from the sources present in the wool. If an appreciable portion of the activity were due to fall-out contamination, we should observe ruthenium-106 and cerium-144 in larger than trace quantities, hence the major portion of it must be due to the second cause. Public Health Service Data[3] of July 1958–July 1960, add some credence to this conclusion. For example, the level of cæsium-137 in milk has varied approximately from 15 to 70 μμc./gm. potassium during this period and now is running in the range 15–25. The observed values for wool are also in this range.

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BIOLOGY

Toxicity associated with Certain Samples of Groundnuts

A new disease, called 'turkey *X* disease' has been described since the widespread outbreaks of deaths in turkey poults in 1960[1]. Post-mortem examination of dead poults from field outbreaks revealed acute hepatic necrosis, associated with generalized bile duct proliferation. Siller and Ostler[2] directed attention to the similarities of the lesions to those of *Senecio*-alkaloid poisoning in the fowl described by Campbell[3].

In the search for the toxic agent, no contamination with any known insecticide, solvent, plant toxin or alkaloid could be found. However, Blount noted that these outbreaks of disease had followed the use of Brazilian groundnut meal in the feed. Some batches of the meal were shown to be toxic for turkeys[1], and for ducklings[4]. A toxic fraction has now been isolated and shown to be free from plant alkaloids[4]. This toxic material has also been found in some samples of other groundnut meals and groundnuts[5].

While the above investigations were in progress, we observed that feeding Brazilian groundnut meal to newly weaned rats did not produce the acute toxicity seen in turkey poults. However, a few weeks of feeding a diet containing 20 per cent Brazilian groundnut meal reduced growth-rate and food intake, and the continued feeding of this diet caused severe liver lesions. This communication describes the changes seen after a sample of Brazilian groundnut meal known to be toxic to turkey poults had been fed to rats for 6 months.

Three groups (9 male and 7 female) of 4-week-old rats from our inbred colony were caged singly and fed on the following diets: purified diet, purified diet with 20 per cent of the starch replaced by Brazilian groundnut meal, and a diet with 20 per cent (control) Indian groundnut meal. The purified diet used is our standard purified diet (Table 1).

All rats received tap-water *ad lib.*, were weighed weekly and their food intakes measured at twice weekly feeding. After 9 weeks, 2 male and 2 female rats from each group were killed for post-mortem. The remainder were killed after 30 weeks feeding; by this time the males had eaten 650 gm. of groundnut meal.

Histological examinations were carried out as follows: Tissues were fixed in formol-saline, embedded in paraffin and sectioned at 7μ (hæmatoxylin and eosin); the liver sections were also stained by Gallegio, reticulin (Gordon and Sweet) and periodic acid–Schiff methods. Frozen sections of the liver and kidney were stained for fat by sudan IV.

Growth-rate, food consumption and food conversion ratio were similar in the two control groups receiving the purified diet and the Indian groundnut meal. The weights of rats fed the Brazilian groundnut meal were all lower than the controls; the difference was not significant for the females, but the males reached a difference of 70 gm. at the end of the test. The food intake and conversion of these rats were also lower than those of the controls (Table 2). No other clinical changes were noted.

Post-mortem examination after 9 weeks. In the rats killed after 9 weeks feeding, macroscopic lesions were confined to the liver of those rats fed Brazilian groundnut meal. The surface of the liver was smooth, with numerous sub-capsular yellow focal

Table 1. Composition of Diets

	Control basal diet (per cent)	Suspect Brazilian meal (per cent)	Control Indian meal (per cent)
Casein + 0·75 per cent L-cystine	20·15	20·15	20·15
Groundnut oil	10	10	10
Starch	57·85	37·85	37·85
Cellulose	6	6	6
Jones Foster salt mixture*	4	4	4
Wheat germ oil	1·5	1·5	1·5
Vitamins (see below)	0·5	0·5	0·5
Groundnut meal	—	20	20
Vitamin A 200 I.U. Vitamin D_3 21·8 I.U.	In groundnut oil once weekly to each rat		

Vitamins (mgm./100 gm. diet): B_{12}, 0·003; folic acid, 1·5; *p*-aminobenzoic acid, 10; biotin, 0·02; inositol, 22; thiamine, 0·5; riboflavin, 0·65; pyridoxine, 0·3; pantothenic acid, 3·0; ascorbic acid, 0·5; nicotinic acid, 60; choline chloride, 400.
* *J. Nut.*, **24**, 245 (1942).

Table 2. Growth and Food Utilization

	Mean food intakes (gm.)				Mean body-weight gains (gm.)				Mean gains (gm.)/food consumed (gm.)			
	Males		Females		Males		Females		Males		Females	
	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks	0–6 weeks	0–9 weeks
Control, purified diet	553·0	886·6	473·2	745·4	164·4	203·3	97·0	111·3	0·297	0·229	0·206	0·150
Brazilian groundnut meal (20 per cent)*	462·5	743·5	423·5	669·2	126·5	161·1	82·1	92·2	0·274	0·217	0·194	0·137
Control, groundnut meal, Indian (20 per cent)	567·6	887·3	482·9	744·4	167·7	208·1	98·2	111·9	0·296	0·235	0·205	0·151

* Significant difference ($P = 0·05$) between results for Brazilian groundnut meal diet and control diets.

Table 3. Effect of Feeding Groundnut Meals on Liver Weight and Tumour Incidence

Group	Average liver weight (gm.)		Liver tumours	
	Male	Female	Male	Female
Purified diet	13·6	8·2	0/7	0/5
Purified diet + Brazilian groundnut meal, 20 per cent	28·9	16·6	5/6*	4/5
Purified diet + control (Indian) groundnut, 20 per cent	17·2	10·2	1/7	0/6
Stock diet (containing 10 per cent groundnut meal)	—	—	0/243	1/257

Rats 6–12 mths. old.
* One death at 6 weeks.

lesions 1–2 mm. diameter. The kidney appeared normal, but sections showed some hydroptic change in the tubules. No macroscopic or microscopic lesions were found in the liver and kidneys of the rats in either of the control groups.

Post-mortem examination after 30 weeks. In the rats killed at the end of 30 weeks feeding, macroscopic lesions were confined to the livers and lungs of the rats fed the Brazilian groundnut meal. The other organs—heart, spleen, kidney, adrenal, intestinal and urogenital tract—all appeared normal. The livers of all rats in this group were grossly abnormal, twice the weight of the controls (Table 3) with a brownish yellow irregular nodular surface, red and greenish cysts and numerous (1–2 mm. diameter) yellowish focal lesions. The nodules consist of liver tumours of three types: solid yellowish lobulated hepatomas, and blood- or bile-filled cysts. Of the 11 rats in the group fed Brazilian groundnut meal, 9 had macroscopic tumours, and two of these rats also had secondary deposits in the lungs. The yellow focal lesions seen in all the livers in this group are similar to those found in the rats killed at 9 weeks.

In the control animals, one hepatoma was found in the group fed the control groundnut meal, but no focal lesions were seen. There were no macroscopic lesions in the rats fed the purified diet.

The kidneys were all macroscopically normal, but in the group fed Brazilian groundnut meal, sections show some glomerular and tubular changes. In some glomeruli there is a homogeneous eosinophilic deposit on the glomerular membrane. Some tubules show a few greatly enlarged hydroptic cells; these have very large vesicular nuclei and much vacuolated cytoplasm. No changes were found in the kidneys of control rats.

The lungs all show various stages of the rat pneumonia complex; however, in two rats fed Brazilian groundnut meal macroscopic greyish lesions 2–3 mm. in diameter with a wide surrounding zone of hæmorrhage were found. Sections show infiltration with anaplastic hepatoma cells, with mitosis, and a wide outer zone of alveoli filled with alveolar phagocytes and red blood cells. Control rats show only the lesions of the rat pneumonia complex.

We have therefore found that the feeding of a known toxic sample of Brazilian groundnut meal to rats failed to reproduce the acute liver damage which occurred in turkey poults. The growth of male and female rats was depressed, but this was accompanied by a reduced food intake and lowered food conversion ratio (Table 2). Diets containing the toxic meal were unpalatable and reduced the growth-rate in turkey poults[1].

After six months feeding of 20 per cent Brazilian groundnut meal in a purified diet, 9 out of 11 rats developed multiple liver tumours, and 2 of these had lung metastases. This finding indicates that this diet is carcinogenic. In comparison the breeding stock show a very low incidence of liver tumours (Table 3).

The presence of liver tumours in the absence of cirrhosis, cell necrosis, or cellular infiltration suggests that the toxic agent acts directly on the hepatic cells.

The nature of the toxic agent is at present unknown; so far all the attempts to show significant amounts of known toxic substances have been negative[1], and in spite of the resemblance of the liver lesions in turkey poults to those produced by *Senecio* alkaloids, these have not been found in the toxic extract[4]. The presence of the toxin in groundnut meals from other sources has been reported[5]; but our finding of a single hepatoma in rats fed the control Indian groundnut meal is not conclusive evidence for the presence in this sample of the carcinogenic agent.

Extracts of the meal are now being tested for their hepatotoxic and carcinogenic effect in rats, but we think that these unexpected preliminary results are of general interest.

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Large numbers of turkey poults[1] and ducklings[2] died on British farms in 1960 as a result of consuming groundnut (*Arachis hypogaea*) meal imported from Brazil. Afterwards, outbreaks of disease associated with the feeding of Brazilian groundnut meal were reported in cattle[3], pigs[4], and sheep (Buxton, J. C., personal communication). More recently it has been shown that some samples of groundnut products from a number of other producing countries are toxic to animals[5].

Toxic groundnuts cause hepatic changes in all the species mentioned. In cattle, the disease has been reported as indistinguishable from ragwort (*Senecio jacobea*) poisoning[3]. In rats fed on a diet containing toxic groundnut meal for 6 months, carcinoma of the liver developed (Lancaster, M. C., *et al.*, preceding communication).

Earlier work[6] provided a sensitive biological method of detecting toxicity in groundnut products and the active substance was concentrated 250 times. We have now further purified the toxic extracts from Brazilian groundnut meal by chromatography on alumina and obtained a crystalline though still not pure substance which produces the same biological effects in ducklings. Twenty μgm. of this material given orally is fatal to day-old ducklings in 24 hr., and considerably less produces the characteristic histological liver lesions.

The toxic substance is almost colourless, and on paper chromatography on Whatman No. 1 paper in *n*-butanol–5 per cent acetic acid gives a single spot, R_F 0·7, which emits a bright blue fluorescence in ultra-violet light. The amount of fluorescent material estimated visually to be present in the spots produced in this way from numerous samples has invariably corresponded with the toxicity of the sample as determined biologically. We are now using this method to select samples for biological testing; when a little more is known of the physical and chemical properties of the toxic material we have every hope that the

method will provide the basis of a routine chemical test.

It was suspected that the toxic substance might be a fungal metabolite since a highly toxic sample of nuts from Uganda which had been associated with the deaths of ducklings in Kenya was seen to be heavily contaminated with fungi.

Pure cultures of certain of the fungal species present in this sample were produced and those of eight isolates, grown for 7 days at 27° C. on Czapek's solution agar, were extracted with chloroform. The extract from one of these was shown to contain a fluorescent material, R_F 0·7, in *n*-butanol–5 per cent acetic acid. These extracts were dosed to groups of day-old ducklings and those receiving the fluorescent extract died with the characteristic histological liver lesions of toxic groundnut poisoning. The dose required was also of the order expected from a consideration of the amount of the fluorescent material present. The toxin-producing fungus was next grown on heat-sterilized, non-toxic groundnuts. After 7 days at room temperature extracts prepared from the visibly mouldy nuts were shown to contain the blue-fluorescent material and to be lethal to day-old ducklings, producing the typical liver lesions.

The toxin-producing fungus has now been identified as *Aspergillus flavus* Link ex Fries.

Further work on several aspects of this problem is now being pursued. Thus the toxic substance is being prepared in a pure state from cultures of *A. flavus* for comparison with the crystalline material isolated from the toxic groundnut meal, and then for detailed chemical and biological study. Examination of other fungi isolated from groundnuts is also being continued to discover whether they produce a toxin. Development of a routine chemical test for the toxin is also in hand, while other sections of the Tropical Products Institute are studying the incidence of fungal attack on groundnuts in the tropics and the steps necessary to prevent it. Finally, we are examining possible economic methods of making toxic meals safe.

Since strains of *Aspergillus flavus* are among the commonest fungal contaminants of cereal grains[7], it would not be surprising if some grain samples were found to be contaminated with the toxic metabolite described. We intend to study this problem.

We wish to thank Mr. J. J. Elphick, of the Commonwealth Mycological Institute at Kew, for identifying the toxin-producing fungus.

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Leaf Vernalization

Vernalization is the indirect stimulating effect of low temperature on subsequent flower formation in high temperatures. So far seed vernalization and plant vernalization are known. In winter annuals vernalization of germinating seed is effective. In biennials seed vernalization has no visible effect, but 'adult' plants—which have passed the juvenile phase—can be vernalized.

In their classical experiments, Gregory and Purvis[1,2] and Purvis[3,4] demonstrated that in *Secale cereale* variety Petkus the locus of seed vernalization lies within the embryo and more specifically in the stem apex. Curtis and Chang[5] found that in celery the terminal meristems of the stem can be vernalized. Further investigations on the locus of plant vernalization are unknown, but it is believed that the stem apices, or the buds, are the only organs that perceive vernalizing temperatures. However, recent experiments have shown that leaves can be also vernalized.

These experiments were carried out with leaf cuttings of honesty, *Lunaria biennis*. According to former investigations[6] this plant is a typical cold-requiring biennial. Hagemann[7] demonstrated that leaf cuttings, consisting of a complete leaf with part of its petiole but without a bud, regenerate easily and reproduce the developmental phase of the plant of origin: leaf cuttings from vegetative plants remain vegetative, but those of generative plants will flower. Unpublished results by me have shown that translocation of a 'flowering stimulus' from vernalized scions to unvernalized stocks may occur.

The experimental approach to leaf vernalization was quite simple. Leaf cuttings of juvenile and adult plants, 6 and 12 weeks old respectively, were obtained. These cuttings were made of leaves of approximately the same age and were exposed to 5° C. during 12 or 20 weeks and afterwards grown at ordinary greenhouse temperatures. None of the cuttings from juvenile plants flowered. Of the adult plants none of the 25 controls flowered, but 3 out of 17 (17 per cent) and 21 out of 24 (88 per cent) after 12 weeks and 20 weeks of vernalization, respectively, flowered quite normally. They had visible flower buds after, respectively, averages of 219 and 182 days from the day of cutting. The cuttings had started root formation while at 5° C., but no bud had yet regenerated. Consequently, vernalization of leaf plus part of petiole without a bud had taken place.

Two additional points are of interest. First—and this is quite remarkable—the juvenile nature of the original plants is maintained in the leaf cuttings. Secondly, leaves from adult plants require more cold than whole plants, as 9 weeks of 5° C. and sometimes less are already sufficient for complete vernalization of 12-week-old plants[6].

Two possibilities offer themselves as explanations of leaf vernalization. (1) The whole leaf is vernalized, resulting in the production of a flowering stimulus which is translocated to the regenerating new plant. The results of Hagemann and of my own grafting experiments tend to support this possibility. (2) Each meristematic cell is subject to vernalization and reproduces the vernalized condition by mitosis to the newly regenerating plant. It is difficult to choose between these possibilities; but experiments are under way to settle this question.

The investigation has been extended by growing the leaf cuttings at ordinary greenhouse temperatures for 2 weeks before the cold treatments started. This was done on the supposition that the cuttings would

stand the cold treatments better. After all, it has turned out to be unnecessary, but rather unexpected results were obtained: percentages of flowering between 72 and 87 per cent in the four groups of 6- or 12-week-old plants and 12 or 20 weeks vernalization. It should be realized that the leaves from the 6-week-old plants were 2 weeks older when the cold treatment started, and according to former results plants of 8 weeks old are usually adult. Furthermore, root formation had started when the cuttings were exposed to low temperature, while a number of them had formed buds during the cold treatment. However, cuttings without buds at the end of the vernalization have flowered, while for the rest the experiments without a 2-week warm period and without buds formed in the cold are conclusive enough. The favourable effect of a preceding root formation remains unexplained.

Finally, some side-results with regard to the influence of low temperature on the regeneration are worth while mentioning. All cuttings formed roots quite readily. All cuttings from 6-week-old plants without cold treatment regenerated sprouts after an average of 89 days. Only 52 per cent of the cuttings from 12-week-old plants without cold treatment regenerated sprouts after an average of 169 days. After cold treatment, however, 100 per cent regenerated sprouts after approximately the same time as the comparable 6-week groups. Judging by the average numbers of sprouts per plant the low temperature had a decidedly favourable effect: 1·3–2·6 in the 6-week group and 1·2–4·8 in the 12-week group.

In conclusion, leaf vernalization is possible when the leaves are taken from adult plants. On the other hand, the regeneration of sprouts is much better when the leaves arise from juvenile plants. Low temperature has a decidedly favourable effect on this regeneration.

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A Potent, Non-Toxic Plant-growth Inhibitor from an Antibiotic Fermentation

The production by micro-organisms of substances inhibitory to the growth of higher plants is well documented. The purpose of this communication is to report a potent inhibitor of plant growth, as measured by our duckweed (*Lemna*) test[1]. Solvent extraction at *p*H 9·0 of filtrates derived from fermentations of an unidentified *Streptomyces* led to the recovery of a light tan solid, designated crude *PA*-180. This preparation exhibited considerable antibiotic activity against various micro-organisms (for example, minimum inhibitory concentrations of 3·12 and 6·25 μgm./ml. against *Streptococcus faecalis* and *Corynebacterium diphtheriae*, respectively; we wish to thank Dr. A. R. English for the anti-microbial testing and Dr. T. M. Lees and Mr. M. Lynch for papergram determinations). This crude *PA*-180 preparation also was shown to exhibit remarkable plant growth activity. This inhibitory effect was first noticed in a routine test being conducted for an evaluation of its growth effect and its phytotoxicity. The results (Fig. 1) were noteworthy since they demonstrated a strong inhibitory action with no apparent toxic symptoms. The plants at all test-levels were normal except for a decreased size. The original experimental transfer consisted of one rosette of four fronds. These four original fronds are easily detected among the tiny daughter fronds at the 5, 10 and 20 p.p.m. test-levels. The number of fronds is not appreciably decreased, as opposed to the size and weight of the plants.

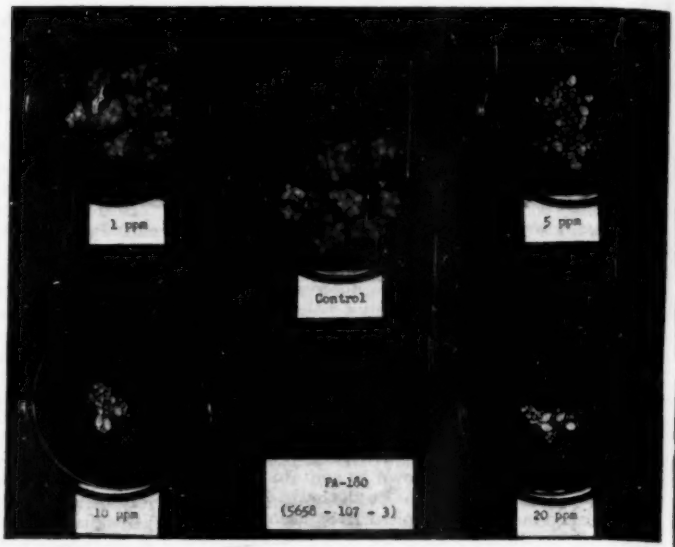

Fig. 1. Effects of a preparation of *PA*-180 on the growth of *Lemna minor* under aseptic conditions on a liquid synthetic medium. The dwarfing effect is apparent at 5 p.p.m. and more. The original four leaves of the rosette used for inoculation are clearly shown at these same levels

The effect of this new inhibitor on the fresh weight of *Lemna minor*, a small aquatic duckweed grown under aseptic conditions on a simple, synthetic liquid medium, is shown in Table 1 along with similar data in this test using several microbial metabolic products, including antibiotics and some well-known plant-growth and general metabolic inhibitors. Present investigations are being conducted for the purpose of determining how widespread this inhibitory action is and the range of plants affected, including the lower forms. Preliminary results show inhibition of: (1) several plant tissues *in vitro*; (2) cucumber seed germination; and (3) growth of barley and several other seedlings.

Efforts were then directed toward the preliminary characterization of crude *PA*-180. Papergram

Table 1. Effect of Fermentation Products (including Antibiotics), Plant-Growth Inhibitors and Metabolic Inhibitors on the Growth of a Duckweed (*Lemna minor*) under Aseptic Conditions

Compound	Percentage change from control (wet weight basis) 1 p.p.m.	5 p.p.m.	10 p.p.m.	20 p.p.m.	Comments
Actidione (refs. 1, 2)	dead	dead	dead	dead	
Anisomycin	−80	−90	−90	dead	leaves small, chlorotic; complete root inhibition
Azaserine (ref. 3)	dead	dead	dead	dead	
Bacitracin (refs. 1, 4, 5)	0	+80	+100	+130	
Catenulin (ref. 1)	dead	dead	dead	dead	
Duramycin (ref. 6)	+35	+35	+35	+30	root inhibition at 20 p.p.m.
Griseofulvin (ref. 1)	−10	−15	−20	...	
Kojic acid	+40	+70	+65	+70	
Oleandomycin phosphate (ref. 5)	0	0	0	0	
Penicillin *G* (refs. 1, 4, 5)	+60	+225	+230	+220	
Penicillin *S*	+100	+210	+290	+240	
Polymyxin *B* (refs. 4, 7)	−15	−15	dead	dead	
Trichomycin	0	+5	+10	+35	
PA-180	−15 to −60	−85	−90	−95	tiny plants at 5–20 p.p.m.
Azauracil	−20	dead	dead	dead	
3-Amino-1,2,4-triazole (ref. 8)	−5	−10	−50	−70	chlorosis and root inhibition at 5–20 p.p.m.
Amo-1618 (refs. 9, 10, 11)	+10	+20	+20	+25	
Amo-1619 (ref. 10)	+15	+15	+10	+10	
2,4-Dinitrophenol (refs. 12, 13)	−25	dead	dead	dead	
(β-Bromoethyl) trimethyl-ammonium bromide (refs. 14, 15, 16)	0	+10	+5	−55	
(β-Chloroethyl) trimethyl-ammonium chloride (refs. 14, 15, 16)	+55	+20	0	0	
(2,3-*n*-Propylene) trimethyl-ammonium chloride (refs. 14, 15, 16)	+40	+25	10	0	
Maleic hydrazide (ref. 17)	0	−10	−25	−40	root inhibition at 10–20 p.p.m.

investigations utilizing various chemical and physical detection techniques disclosed a heterogeneous nature for the material. Bioautographs showed the presence of one dominant antibiotic substance. Work with eluates of papergram sections (with several culture strains) led to detection of plant-growth inhibitory activity dissociated from that of the dominant antibiotic component; heat stability of the plant factor was also demonstrated. It is apparent that the plant factor is a small but potent fraction of crude *PA*-180. Thousands of materials have been screened to date in this test, but none has demonstrated the amount, type and reproducibility of plant-inhibitory activity associated with crude *PA*-180, with the possible exception of another actinomycete fermentation preparation (at present being compared with the *PA*-180 product).

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Antennary Feeding of the Hermit Crab *Diogenes brevirostris* Stimpson

There exist only two previous descriptions of feeding behaviour in hermit crabs, namely, those of Thompson, who described the feeding of *Eupagurus longicarpus* Say., and of Orton on *E. bernhardus* (L.). Orton[1] says that both species feed by scooping up debris directly with either the third maxillipeds or with the small chela. The material is brushed between the third maxillipeds, and the rejected portion falls to the ground. The antennæ and the walking legs play no part in the feeding of these animals.

Diogenes brevirostris Stimpson usually feeds by scraping debris towards the third maxillipeds with the second and third walking legs, and the small chela. The material is picked up by the endopods of the third maxillipeds, scrubbed between the brushes of the propus, dactylus and merus, and then passed on to the endopods of the second maxillipeds. These brush the debris further and pass the food to the mouth, the inedible portions being carried away in the exhalent respiratory current. Small pieces of algæ may be picked from stones by the small chela and passed on to the mouth parts and so to the mouth.

The alternative method of feeding can be described as antennary cast-net feeding. The flagellum of each antenna bears two rows of plumose setæ. These are about 3 mm. long and set widely apart, each being separated from its neighbour by 0·2 mm. The setæ of the two rows curve in towards one another so as to form an obtuse and reflex angle between them. The animals normally make occasional cleaning movements of the antennæ, bringing them down alternately to the endopods of the third maxillipeds, which clean them by wiping off the debris caught on the setæ. The antennæ then resume their normal resting position, the oblique angle between the setæ facing downwards and slightly laterally. If, however, food particles are caught on the setæ, the antennæ begin rapid to-and-fro movements. These are interspersed with cleaning movements after every three or four sweeps, and the adhering particles are removed by the maxillipeds and eaten. During each sweep the antennæ are twisted so that the oblique angle between

the setæ faces in the direction of the sweep. If the animals are feeding in a water current the antennæ are not swept, but merely kept with the oblique angle between the setæ facing into the oncoming current so as to catch the suspended particles. The antennæ are then cleaned in the normal manner. Sorting of the edible and inedible fractions takes place in the usual manner.

Cast-net feeding occurs in other Crustacea, but appendages other than the antennæ may be used. Thus *Porcellana longicornis* (L.)[2] and *Haplocarcinus marsupialis* Stimpson[1] use the third maxillipeds as cast-nets. The use of antennæ as cast-nets is found in the nauplius larvæ of *Artemia salina* (L.)[3], while two anomuran sand crabs, *Emerita talpoidea* (Say), and *E. analoga* Stimpson, are also known to feed with their antennæ. They feed in the wash of the waves on the shore, with their antennæ extended as nets. It is reported that *E. analoga* can feed in quiet water by waving its antennæ, but that *E. talpoidea* cannot feed in quiet water at all[4]. There is, however, a distinct difference between the structure of the antennæ of *Emerita* and *Diogenes*. The flagellum of each antenna of *E. austroafricana* Schmitt has two double rows of setæ, the outer setæ of each pair bearing long setules, and the inner short setules. These crabs depend almost exclusively on cast-net feeding, and correlated with this carry on their antennæ a dense feltwork of setules which can trap both large and minute particles. This is not true of *Diogenes brevirostris*, which uses its antennæ only as accessory feeding devices.

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Spawning Mechanism in the Male Lugworm

It has been shown[1] that the male lugworm (*Arenicola marina*, L.) can be induced to spawn by injecting into the body-cavity the lipid fraction of tissue extracts of whole worms. It was suggested that release of lipid into the body cavity might provide the immediate (internal) stimulus for spawning under natural conditions. The validity of this suggestion can only be tested when the mechanism by which the gametes are emitted is thoroughly understood.

In the lugworm the gametes develop in the body-cavity for a prolonged period and are eventually shed through the nephromixia[2]. The problem in the elucidation of the spawning mechanism lies in the fact that during their development the gametes must be rejected by the ciliated funnels of the nephromixia so that they are retained in the body-cavity, whereas when spawning commences they must suddenly be accepted by the ciliated funnels so that they are shed.

The answer to this problem suggested itself in the experiments, mentioned above, in which tissue extracts were injected into male worms. It was noted that before the worms began to spawn the spermatozoa in the cœlom, which are normally bound together in morulæ, became dissociated forming a dense suspension of free spermatozoa. It seemed possible that whereas sperm morulæ were rejected by the nephromixia, free spermatozoa were accepted by them so that shedding automatically ensued. This possibility has since been tested in a series of experiments in which samples of free spermatozoa were injected into the body-cavities of male worms which were not themselves spawning. Samples of free spermatozoa were obtained by concentrating sperm suspensions spawned naturally by centrifugation, or by treating cœlomic sperm *in vivo* or *in vitro* with lipid extract. The lipid was afterwards separated, again by centrifugation. As it could be argued that free spermatozoa obtained by both these methods might be contaminated with a natural spawning stimulant, samples of free sperm were also obtained by treating cœlomic sperm with alkali. The *p*H of the suspension was afterwards adjusted to 7·5. Untreated cœlomic sperm was administered to control worms. 1·0–1·5 ml. of one of these concentrated suspensions was administered to each test worm, the total number of free sperm injected having been estimated previously by counts using a 'Metz' hæmocytometer. With the exception of the controls, shedding ensued in every case; usually less than 1 hr. after injection of the free spermatozoa (Table 1). It would appear, therefore, that free spermatozoa are accepted automatically by the nephromixia and shed. It is possible, however, that shedding under natural conditions is not entirely passive. In these experiments spermatozoa were shed from the pores of the nephromixia much less vigorously than in normal spawning. Furthermore, estimates of the number of free sperm remaining in the body cavities of the test worms after shedding had ceased showed that in most cases only a third to a half of the total free sperm injected had been shed (Table 1).

Table 1. Counts by Hæmocytometer of Free Sperm injected into Male Worms and of the Number remaining in the Body Cavity after Cessation of Shedding

Worm No.	Type or treatment of injected sperm	Total free sperm ($\times 10^6$) injected	Spawning occurred in (+)	Time of spawning (min.) after injection	Total free sperm ($\times 10^6$) in body cavity after spawning ceased
1	Naturally spawned	2·0	+	24	0·3
2	Naturally spawned	3·1	+	10	not counted infected
3	Naturally spawned	3·1	+	28	not counted infected
4	Extract (*in vitro*)	8–10·3	+	90	3·2
5	Extract (*in vitro*)	8–10·3	+	overnight	8·4
6	Extract (*in vitro*)	8–10·3	+	60	5·3
7	Extract (*in vivo*)	8–12·6	+	28	2·9
8	Extract (*in vivo*)	8–12·6	+	14	3·0
9	Extract (*in vivo*)	8–12·6	+	13	2·5
10	Alkali (*in vitro*)	11·0	+	9	4·1
11	Alkali (*in vitro*)	11·0	+	overnight	10·9
12	Alkali (*in vitro*)	11·0	+	15	4·1
13	Control untreated	1·75	—	—	—
14	Control untreated	1·75	—	—	—
15	Control untreated	2·0	—	—	—
16	Control untreated	2·0	—	—	—

While no great accuracy can be claimed for these counts due to difficulties in estimating the volume of the cœlomic fluid in each worm, it is clear that substantial numbers of free spermatozoa were usually retained in the body cavity. Even in the controls substantial numbers of free sperm were present in the suspension injected (due to handling of the suspension and to the fact that it was exposed to the air for some time) and none of this sperm was shed. This suggests that in normal spawning there is more active uptake of free sperm from the body-cavity. On the other hand, it was noticeable that when concentrated suspensions of naturally spawned sperm were injected

there was more complete evacuation of free sperm from the body cavity although it was only possible to make one count (Table 1). Furthermore, when a microscopic examination was made of the suspensions of free spermatozoa produced by treatment with extract and alkali, it did appear that although the sperm morulæ had been broken down there was a tendency for the spermatozoa to continue to adhere together by their tails. It may have been for this reason that many apparently free spermatozoa were not shed whereas the naturally spawned sperm was completely dissociated and thus accepted more readily by the nephromixia. The correct interpretation of this evidence was suggested in similar experiments with female worms, which are reported at greater length elsewhere[3]. In these it was shown that maturation of the ova immediately precedes the onset of shedding. This suggested that mature eggs might be accepted by the nephromixia whereas immature eggs are rejected. Mature eggs which had been spawned naturally were collected and concentrated. As in the experiments with males, these were injected into test worms which were not spawning. Shedding immediately ensued, but again only about half of the material injected was emitted. It appears, therefore, that emission of the gametes in *A. marina* involves primarily a change in the nature of the gametes which is associated with their maturation so that they can be accepted by the ciliated funnels of the nephromixia; and secondly, there may also be some form of active uptake of the gametes by the nephromixia.

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VIROLOGY

Cytopathic Protective Factor: its Production by Normal Uninfected Serum-free Cell Cultures

At the present time two well-defined systems of viral interference by cell products have been demonstrated *in vitro*. They are interferon[1] and viral inhibitory factor[2], the latter possibly being a variety of interferon. Both at least have in common the fact that they are produced in cell cultures infected with virus, active virus in the case of the latter, or inactivated where interferon is concerned. Other differences and similarities are discussed in a recent report by Ho and Enders[3]. It is well known that virus inhibitors are present in a variety of mammalian tissues and fluids[4] and may be demonstrated either *in vivo* or *in vitro*.

In the process of searching for biologically synthesized inhibitors for viruses, it was discovered that a line of serum-free mouse lung cells produced a substance which 'protected' heterologous cells from destruction by influenza virus. The lung cells were originally isolated from lung tissue of new-born white Swiss mice on a medium of 10 per cent horse serum and 90 per cent medium 199. They were afterwards adapted to rapid growth on a medium consisting of 1 per cent peptone, 100 mgm. per cent dextrose, and 99 per cent medium 199 (ref. 5). They have been designated the *MPD*1 line of cells. It had also previously been shown that the serum-free cells were equally as susceptible to vaccinia virus infection as the original serum-containing parent strain[6]. Neither line of cells showed any response to dilute or undilute influenza viruses *PR*8, Asian, Lee *B*, or *B*54, when 40-hr. or older cultures were used.

Two additional cell lines, one designated *UIRHM*1 (University of Illinois rabbit heart mark 1) isolated from normal rabbit heart tissue, and one designated *UIKM*1 (University of Illinois keratoacanthoma mark 1) isolated from a human keratoacanthoma lesion[7], are fully susceptible to all four of the influenza viruses previously mentioned. Both these cell lines contained 10 per cent calf serum as a supplement to the base medium 199. The cytopathic effect of the virus was evidenced as a pronounced cytoplasmic vacuolation with eventual cell destruction in the *UIKM*1 cells. In the case of the *UIRHM*1 cells the destruction of cells occurred without cytoplasmic vacuolation. These cytopathic effects could be prevented by specific antiserum, and occurred within 48 hr. of virus inoculation.

Following the basic recognition that such a phenomenon occurred with *MPD*1 fluids and influenza virus, it was decided to develop and control an investigation into this 'protective' effect.

Cultures of *UIRHM*1 and *UIKM*1 cells were seeded in a volume of 5×10^5 cells per ml. in 1-ml. Leighton tubes and incubated at 37° C. for 24 hr. At the end of this time, the supernatant fluid was removed and the cells were covered with 1 ml. of pooled 6-day supernatant fluids from *MPD*1 cells. 5 min. after the addition of the 6-day old fluid, the cultures were inoculated with 0·05 or 0·1 ml. of a specific strain of influenza virus, either *PR*8 or *B*54, which had been grown in chick embryos. Controls consisted of normal cultures, cells which were reared with maintenance media (99 per cent Hanks balanced salt solution and 1 per cent calf serum), and cells to which complete fresh peptone 199 media was added. In addition to these, a third series of tubes received an overlay of pooled *MPD*1 supernatant fluid to which had been added concentrated medium 199 and calf serum so that the amount of these substances present was equal to their original concentration in the test cell media. The cells were re-incubated at 37° C. and viewed microscopically at 17, 24, and 40 hr. The cytopathic response was graded as 0, 1+, 2+, 3+, or 4+. Under this system, 0 would indicate no cytopathic response or complete cell protection, 1+ as 25 per cent, 2+ as 50 per cent, 3+ as 75 per cent, and 4+ as 100 per cent cytopathic effect.

Results showed complete protection against cytopathic effect of both influenza viruses in the two cell lines. In addition, it was found that this same protection occurred when Lee *B* or Asian influenza viruses were used. This protective fluid was afterwards named cytopathic protective factor.

In order to standardize future work, a test system composed of *UIRHM*1 cells and *B*54 virus was chosen. The standard inoculum was approximately 3×10^5 tissue cells per ml. of fluid and 0·1 ml. of *B*54 chorioallantoic fluid virus, containing $10^{7.1}$ 50 per cent chick embryo infective doses (EID_{50}).

In order to determine the concentration of cytopathic factor in the supernatant fluids, the following experiments were set up. Fluids from pooled 6-day harvests of *MPD*1 cells were diluted from 1 : 2 up to 1 : 512 in two-fold steps in complete growth media. The fluid from 24–72 hr. old *UIRHM*1 cells was decanted and the cells overlayed with specific cyto-

pathic factor dilutions, and the standard testing procedure carried out. Four tubes were used for each dilution, as well as the normal control series. It was possible to dilute the cytopathic factor 1 : 16 and still protect 75 per cent of the cells. A dilution of 1 : 8 afforded 100 per cent cell protection.

At this point it was considered vital to know whether the cytopathic factor acted on the cell *per se* or the virus particle. To resolve this problem, the fluid from 24-hr. old cultures of *UIRHM*1 cells was decanted and the cell sheet was overlayed with undiluted cytopathic factor fluid and incubated for 1, 3, 6, and 20 hr. at 37° C. At the end of this time, the fluids were removed and replaced by complete growth media and 0·1 ml. of *B*54 virus. No protection occurred at any of the periods tested, showing that the cytopathic factor was not adsorbed to the cell sheet.

To test the action of cytopathic factor on influenza virus, *B*54 virus was diluted 1 : 10 in the undiluted factor fluid and incubated at 37° C. for 1, 3, 6, and 20 hr. At the end of this time, the virus was sedimented by centrifugation at 81,000*g* for 30 min. The fluid was decanted and the virus re-suspended, to its original stock concentration, in complete growth media and used in the standard test system. The virus was fully as toxic to *UIRHM*1 cells as untreated virus, indicating that the action of cytopathic factor is not directed against the virus *per se*. The lack of action on the virus was further demonstrated by its negative reaction in hæmagglutination inhibition tests.

In order to determine the period of formation of cytopathic factor, the following experiment was designed. Six 10-ml. flasks of *MPD*1 cells were inoculated with 4×10^5 cells per ml. and incubated at 37° C. At the end of 24 hr., and every 24 hr. for a total of 6 days, the entire 10 ml. of supernatant fluid was harvested and stored frozen. When all fluids had been collected, they were tested for the presence of cytopathic factor by the dilution method mentioned. The fluid was negative for protective capacity at 24 hr., became positive at 48 hr., and increased to a maximum at 5 days. The period between 24 and 72 hr. is undergoing further investigation.

Present results indicate that the cytopathic factor differs mainly from interferon and viral inhibitory factor in that it is produced in uninfected cultures and not as a result of contact with viruses, either active or inactive. It is similar to viral inhibitory factor in that it neither acts on the virus *per se* nor is adsorbed to the cell sheet. Interferon, on the other hand, is readily bound by test cells. The cytopathic factor appears to be a natural cell product protecting against the cytopathic effect of influenza virus in the cells from which it originates as well as cells from human and rabbit sources. Further, it has not been found in the parent cultures containing serum of the *MPD*1 line. Since both virus infection and adaptation to serum-free media must represent a traumatic experience to the cell, there is the possibility that other manipulations of this type will produce similar materials. That the protection crosses species barriers at a cellular level is not startling, since it has been shown by Alfred and Pumper[8] that the supernatant fluid from *MPD*1 cells or purified portions of it can act as a serum replacement for human liver cells *in vitro*.

Cytopathic factor had been used in attempts to protect the *UIKM*1 line of cells against coxsackie, mumps, vaccinia, poliomyelitis, and adenoviruses to which this cell line is susceptible, without success. Thus it appears to be specific for influenza viruses. Experiments to determine its physical and chemical properties and site of action, as well as work on its purification, are in progress.

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MISCELLANY

The Grayling Fish and River-Names

The grayling fish belongs to the family Thymalidae and is closely related to the family Salmonidae. It offers, I believe, a new and tenable explanation for the much-disputed origin of the river-names 'Thames' and 'Humber'.

When, in British prehistory, these Rivers were so named, fishing was a vital industry, certainly more so than agriculture, and possibly more than hunting. Our rivers were, in those remote days, teeming with fish, and especially fish of the salmon family, so that nothing could be more natural than that some of these rivers should be named 'grayling-river'.

'Grayling' is a name of germanic origin (cf. German: *grau*). But in medieval English the grayling fish retained two names of non-germanic origin: *thyme* and *umbre*. The 'thyme' word for a grayling is due to the fact that the fish has a smell closely resembling that of the herb of the same name. The word is related to Latin *thymallus* (a fish smelling of thyme) and to Greek θύμος, the herb 'thyme' (θύω, 'to smell'). The 'umbre' word for a grayling is due to its grey and shadowy appearance, and may be cognate with, if not directly descended from, Latin *umbra*, 'shade' (*umbra* was a Roman word for a grayling fish).

In his book, *Der Flüssname Thämse unde seine Sippe* (1941), Prof. Max Forster reaches the conclusion that the meaning of 'Thames' is 'dark river'. For 'Humber', Prof. Eilert Ekwall (*Oxford Dictionary of English Place Names*) suggests that the meaning is 'good-river'.

However, I submit that there is a *prima facie* case for expert investigation of the hypothesis that both the Thames and the Humber, and the groups of river-names associated with them, are so called because of the grayling fish that frequented them—the Thames on account of the smell, and the Humber on account of the colour.

If this is acceptable, it has some bearing on the origins of the language spoken by the natives of Britain before the Romans arrived.

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FORTHCOMING EVENTS

Monday, December 18

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. F. J. D. Taylor: "Large Microwave Steerable Aerials for Communication with Artificial Earth Satellites and Space Probes".

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Meeting on "The Mothproofing of Wool".

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION AND TRAINING GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Teaching of Machine Tool Technology as an Academic Subject".

Tuesday, December 19

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. K. G. King: "The Use of Controllable Semiconductor Rectifiers in Machine Control".

Wednesday, December 20

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Scientific Papers.

ROYAL SOCIETY OF MEDICINE, COMPARATIVE MEDICINE SECTION (at 1 Wimpole Street, London, W.1), at 5 p.m.—Meeting on "The Provision and Use of Pathogen-free Laboratory Animals". Speakers: Dr. W. Lane-Petter, Dr. D. G. Davey and Dr. A. O. Betts.

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. R. L. F. Boyd: "Satellite Instrumentation".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Discussion of recently published papers.

Thursday, December 21

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. B. V. Derjaguin (U.S.S.R.) and Mrs. N. D. Shukakidse (U.S.S.R.): "Dependence of the Floatability of Antimonite on the Value of Zeta-Potential"; Dr. J. D. Peacock and Mr. R. Williamson: "Radon Determination as a Prospecting Technique".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. G. W. Mackey: "Some Connections between Ergodic Theory and Group Theory".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN MATHEMATICS—The Principal, College of Education, St. Andrew Street, Aberdeen (December 26).

ORGANIC CHEMIST or BIOCHEMIST (with a good honours degree or equivalent qualification), for research in structural carbohydrate chemistry and immunochemistry—The Secretary, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1 (December 30).

CHAIR OF APPLIED PHYSICS—The Registrar, The University, Hull (December 31).

CHAIR OF SOCIOLOGY—The Registrar, The University, Hull (December 31).

LECTURER IN GEOGRAPHY; a LECTURER IN PHYSICS; and a LECTURER IN PSYCHOLOGY at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

LECTURER or SENIOR LECTURER IN GENETICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

LECTURER (with an honours degree in biochemistry or chemistry with subsequent research and/or teaching experience in some branch of biochemistry) IN BIOCHEMISTRY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

LECTURER (with some experience in computing, preferably in connexion with electronic machines) IN COMPUTING—The Registrar, University College of Wales, Aberystwyth (December 31).

READER or SENIOR LECTURER IN MATHEMATICS at the University College of Townsville, University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

ASSISTANT IN BACTERIOLOGY (Science)—The Secretary of University Court, The University, Glasgow (January 3).

LECTURER IN THE ELECTRONIC COMPUTATION LABORATORY OF THE APPLIED MATHEMATICS DEPARTMENT—The Registrar, The University, Sheffield (January 6).

LECTURER or SENIOR LECTURER IN STATISTICS IN THE DEPARTMENT OF MATHEMATICS—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (January 6).

LECTURER or ASSISTANT (preferably with a special interest in solid state physics) IN PHYSICS—The Secretary, Queen's College (University of St. Andrews), Dundee (January 8).

LECTURER (preferably candidate who can teach in the fields of Romano–British and/or Medieval English archæology) IN ARCHÆOLOGY—The Registrar, The University, Nottingham (January 13).

CHAIR OF ANATOMY in the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

LECTURERS IN PHYSICS—The Registrar and Secretary, King's College (University of Durham), Newcastle-upon-Tyne 1 (January 15).

RESEARCH FELLOW (preferably with experience in elementary particle physics) IN THE DEPARTMENT OF THEORETICAL PHYSICS, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (January 15).

SENIOR DEMONSTRATOR (with a science degree with a major in geology, and special qualifications and research experience in geomorphology) IN GEOMORPHOLOGY IN THE DEPARTMENT OF GEOLOGY—The Registrar, University of Melbourne, Parkville, N.2, Victoria, Australia (January 15).

SENIOR LECTURER (with special qualifications in analysis) IN MATHEMATICS; and a LECTURER and an ASSISTANT LECTURER (with research experience in mathematics) IN MATHEMATICS IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of North Wales, Bangor, North Wales (January 18).

PROFESSOR (with a higher degree, preferably a doctorate, and research in the field of philosophy) OF PHILOSOPHY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 20).

LECTURER IN MATHEMATICAL STATISTICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 22).

READER IN PHYSICS at St. Bartholomew's Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 22).

CHAIR OF THE HISTORY OF SCIENCE AND TECHNOLOGY at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 25).

TURNER AND NEWALL RESEARCH FELLOW (with two or three years postgraduate experience, or industrial experience, and preferably some knowledge of semiconductor devices) IN APPLIED SCIENCE, for research concerned with novel types of electrical machines of special significance to control engineering—The Registrar, University College of North Wales, Bangor, North Wales (January 25).

LECTURERS (2) IN PURE MATHEMATICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 26).

CHAIR OF ENGINEERING in the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, January 31).

DEMONSTRATORS (graduates with some research experience) IN THE DEPARTMENT OF ORGANIC CHEMISTRY—The Registrar, The University, Liverpool, quoting Ref. CV/575/T (January 31).

LECTURER IN PHYSICS—The Registrar, The University, Hull (January 31).

OFFICIAL FELLOW AND TUTOR IN PHYSICS, or a LECTURER IN PHYSICS—The Principal, St. Hugh's College, Oxford (February 1).

READER IN MATHEMATICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 17).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, PHYSICS, METALLURGY, ENGINEERING, GEOLOGY, AGRICULTURAL SCIENCE or related subjects—The Registrar, University of Wales, Cathays Park, Cardiff (March 5).

LECTURER (with a good honours degree in physics with chemistry as a subsidiary subject, and a Diploma in Education or Teachers' Certificate; or a good general degree with physics and chemistry, and at least five years teaching experience) IN PHYSICS with CHEMISTRY in Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. G.13/3.

PSYCHOLOGIST with a Research Unit engaged on laboratory and field studies of complexity of industrial tasks—H. Murrell, Department of Psychology, The University, 22 Berkeley Square, Bristol 8.

RESEARCH FELLOW (Senior or Junior) (with a Ph.D., and preferably postdoctoral research experience), for work on biochemical activities of intact intestine and of isolated subcellular fractions of intestinal mucosal cells—Dr. J. W. Porteous, Department of Biological Chemistry, Marischal College, The University, Aberdeen.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

World University Service. Student Mental Health: Report of a Conference held at Campbell Hall, London, 8th–9th April, 1961. Pp. 44. (London: World University Service, 59 Gloucester Place, 1961.) 2*s.* 6*d.* [259

Institute of Personnel Management. Personnel Management Salaries: Report of a Survey undertaken in February/March 1961. Pp. 32. (London: Institute of Personnel Management, 1961.) 21*s.* [259

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Research Institute, Animal Virus Diseases, Pirbright. Report for the years 1956–1960. Pp. 52. (Pirbright, Surrey: Research Institute, Animal Virus Diseases, 1961.) 2s. 6d. [259

Agricultural Research Council. A Bibliography of Farm Buildings Research 1945–1958. Part 6: Buildings for the Processing and Storage of Fodder. Pp. 52. (London: Agricultural Research Council, 1961.) 4s. [259

Corrosion Science, Vol. 1, No. 1 (August, 1961). (An International Journal of the Science of Corrosion and Protection of Metals.) Pp. 76. Annual subscription: (A) For libraries, government establishments, and research institutions 140s.; 20 dollars. (B) For individuals who write directly to the publisher certifying that the journal is for their personal use 70s.; 10 dollars. (London and New York: Pergamon Press, 1961.) [259

Ministry of Agriculture, Fisheries and Food; Department of Agriculture and Fisheries for Scotland; Department of Health for Scotland. Milk Powder. Pp. v+18. (London: H.M. Stationery Office, 1961.) 1s. 3d. [259

The Royal Institute of Chemistry. Lecture Series, 1961. No. 1: Atmospheric Photochemistry. By Sir Harrie Massey and A. E. Potter. Pp. 27. 5s. No. 2: A Chemist's Introduction to Statistics, Theory of Error and Design of Experiment. By Dr. D. A. Pantony. Pp. ii+38. 5s. No. 3: Recent Development in Polarography. By Dr. G. W. C. Milner. Pp. ii+41. 5s. (London: The Royal Institute of Chemistry, 1961.) [259

Building Research Station Digest (Second Series), No. 14: Concrete Mix Proportioning and Control—2. Pp. 4. (London: H.M. Stationery Office, 1961.) 4d. [259

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World Health Organization. Technical Report Series. No. 219: Arthropod-Borne Viruses—Report of a Study Group. Pp. 63. 3 Swiss francs; 5s.; 1 dollar. No. 223: Programme Development in the Mental Health Field—Tenth Report of the Expert Committee on Mental Health. Pp. 56. 2 Swiss francs; 3s. 6d.; 0.60 dollars. World Directory of Dental Schools. Pp. 228. 15 Swiss francs; 25s.; 5 dollars. (Geneva: World Health Organization; London: H.M. Stationery Office, 1961.) [259

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United States Department of the Interior: Geological Survey. Professional Paper 386-A: Temperature Rise Within Radioactive Liquid Wastes Injected Into Deep Formations. By Herbert E. Skibitzke. Pp. iii+8. 15 cents. Professional Paper 387-A: Botanical Evidence of the Modern History of Nisqually Glacier, Washington. By Robert S. Sigafoos and E. L. Hendricks. Pp. iii+20. Professional Paper 411-A: A Solution of the Differential Equation of Longitudinal Dispersion in Porous Media. By Akio Ogata and R. B. Banks. Pp. iii+7. 15 cents. (Washington, D.C.: Government Printing Office, 1961.) [259

Publications of the Dominion Observatory, Ottawa. Vol. 22, No. 4: Bibliography of Seismology, Items 10514–11580, July–December, 1958. By J. H. Hodgson and F. E. Langill. Pp. 87–133. (Ottawa: Queen's Printer, 1961.) 25 cents. [259

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United States Department of the Interior: Geological Survey. Water-Supply Paper 1330-E: Water Requirements of the Copper Industry. By Orville D. Mussey. Pp. iv+181–219+plate 2. Water-Supply Paper 1706: Surface Water Supply of the United States, 1960. Part 3-B: Cumberland and Tennessee River Basins. Prepared under the direction of E. L. Hendricks. Pp. vii+234. Professional Paper 316-E: Regional Gravity Survey along the Central and Southern Wasatch Front, Utah. By Kenneth L. Cook and Joseph W. Berg, Jr. Pp. iii+75–89+plate 13. (Washington, D.C.: Government Printing Office, 1961.) [410

Meddelanden från Statens Skogsforskningsinstitut, Band 49, 1960-1961. (Reports of the Forest Research Institute of Sweden, Vol. 49.) Pp. iv+580. Flygblad Nr. 74: Skogsträdens Fruktsättning År 1960. Av. E. Fall. Pp. 4+10 figures. Flygblad Nr. 75: Skogsträdens Fruktsättning År 1960. Av. E. Fall. Pp. 4+10 figures. (Stockholm: Statens Skogsforskningsinstitut, 1961.) Skogsforskningsinstitut, [410

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